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# THE
# JOURNAL OF
# EXPERIMENTAL MEDICINE

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# THE EFFECT OF EOSIN UPON TETANUS TOXIN AND UPON TETANUS IN RATS AND GUINEA-PIGS.

BY SIMON FLEXNER, M.D., AND HIDEYO NOGUCHI, M.D.

(*From the Rockefeller Institute for Medical Research, New York.*)

The study of the action of certain photodynamic substances upon living cells, ferments, and toxins has brought out highly interesting facts. The literature upon this subject is already large and will be briefly reviewed in the paper of one of us (Noguchi) upon the action of these chemicals upon snake-venom.[1] Jodlbauer and v. Tappeiner[2] studied the action of certain fluorescent substances upon diphtheria and tetanus toxins. They found that when the toxins were mixed with 0.05 per cent. of eosin and exposed for three days to diffuse sunlight their toxicity had greatly diminished. The sample of tetanus toxin thus treated produced in 1 to 10 m. l. d. local tetanus only, while in 25 m. l. d, and over it was fatal. Huber[3] stated that the addition of one per cent. of eosin to tetanus toxin, and an exposure of six hours to the sunlight, suffice to destroy the poison, but if the mixture is kept in the dark the poison is not deprived of its toxicity. The dose used by Huber in testing toxicity was 1 m.l.d. Huber also noted a loss of hæmolytic power.

Our experiments with tetanus toxin were begun last spring and were completed before Huber's paper appeared. They embrace the study, first, of the influence of certain aniline dyes upon tetanolysin; second, the effect of eosin upon tetanospasmin; and, third, the influence of eosin upon the course of experimental tetanus in rats and guinea-pigs.

## *The Effects of Certain Aniline Dyes upon Tetanolysin.*

Ehrlich[4] showed tetanus toxin to be composed of two poisons—the hæmolysin, tetanolysin, and the convulsive body, tetanospasmin. Madsen,[5] proved the former to be a highly labile body. Our experiments were made with two samples of the toxin: one a dried ammonium sulphate precipitate of bouillon cultures, and the other a fresh filtrate of bouillon cultures. The first was used as a four per cent. solution; the second in full strength, sterile salt being added to produce isotonicity. The determination of initial hæmotoxicity gave the following:

---

[1] To appear in the next number of this Journal.

[2] Jodlbauer and v. Tappeiner, *Münch. med. Wochenschrift*, 1904, li, 737.

[3] Huber, *Arch. f. Hygiene*, 1905, liv, 53.

[4] Ehrlich, *Berliner klin. Wochenschrift*, 1898, xxxv, 273.

TABLE I.

| | SAMPLE I. | SAMPLE II. |
|---|---|---|
| 0.2 c.c. | C. H | C. H. |
| 0.15 " | " | " |
| 0.1 " | " | " |
| 0.07 " | " | Almost C. H. |
| 0.05 " | Almost C. H. | " |
| 0.04 " | Moderate H. | Moderate H. |
| 0.03 " | Slight H. | " |
| 0.025 " | Trace H. | Trace H. |
| 0.02 " | " | " |
| 0.015 " | " | " |
| 0.01 " | No H. | No H |
| 0.007 " | " | " |
| 0.005 " | " | " |

To two cubic centimeters of each preparation varying amounts of one per cent. eosin in saline were added; the mixtures were kept at 37° C. for one hour and tested against two per cent. washed rabbits' corpuscles. The tubes were incubated four hours at 37° C. and kept eighteen hours at room temperature.

TABLE II.

| TETANOLYSIN 2 C.C. | SAMPLE I. | SAMPLE II. |
|---|---|---|
| Eosin 1 % 1.0 c.c. | No H. | No H. |
| " 0.7 " | " | " |
| " 0.5 " | " | " |
| " 0.3 " | " | " |
| " 0.2 " | Slight H. | Slight H. |
| " 0.15 " | " | " |
| " 0.1 " | Moderate H. | Moderate H. |
| " 0.07 " | Much H. | Much H. |
| " 0.05 " | Almost C. H. | Almost C. H. |
| " 0.03 " | C. H. | C. H. |
| " 0 " | C. H. | C. H. |

---

[5] *Zeitschr. f. Hygiene*, 1900, xxxii, 214.

Two cubic centimeters of tetanolysin (representing about 150 m. h. d.[6]) are completely destroyed by 0.3 cubic centimeter of a one per cent. solution of eosin, in one hour in the dark, at 37° C. In other words, an eosin strength of 0.13 per cent. quickly destroyed the lysin.

Simple exposure of tetanus toxin in test-tubes to sunlight for six hours completely destroyed the lysin; and an exposure for two hours in the presence of 0.03 cubic centimeter eosin solution to two cubic centimeters of the toxin also destroyed the hæmolysin. In the dark this small quantity of eosin has no action upon the lysin. The action of the eosin in the light is very rapid, since after ten minutes' exposure the hæmolytic property may be lost.

A series of experiments were made with Grübler's methylene blue, orange G, orcein, fuchsin, and vesuvin. Berlin blue was also tried. The results may be summarized as follows: In strengths of 0.2 per cent. and above, methylene blue and vesuvin destroy tetanus hæmolysin in one hour. Quantities below 0.1 per cent. are partially destructive only; while 0.04 per cent. no longer checks hæmolysis. Fuchsin, orcein, and fluorescein are also injurious, but their limits of action were not determined. Berlin blue is without effect.

These experiments bring out the fact that aniline dyes, fluorescent ones, chiefly, have an injurious effect on the labile hæmolysin in the dark, although the effect is less than in the light.

### *The Effect of Eosin upon Tetanospasmin.*

The fatal constituent of tetanus toxin is the convulsive agent tetanospasmin, which has an especial affinity for nervous tissues. We tested the action of eosin in the dark as well as in the light upon the convulsive principle. To one cubic centimeter of the toxin (Sample II) were added varying amounts of five per cent. eosin. The mixtures were kept one hour at 37° C. before injection in the right gluteal region into white rats, weighing about 90 grams each. The animals were kept in a room diffusely lighted; the cage was partly shaded. The toxin employed was fatal in four days in a dose of 0.0025 cubic centimeter, the first symptoms of tetanus appearing in twenty-four hours; 0.0005 cubic centimeter was the minimal tetanic dose, and 0.001 caused strong tetanus but was not fatal. Doses of about 0.4 cubic centimeter caused death in from 24 to 48 hours. Hence one cubic centimeter of the toxin contained about 400 m. l. d. and 2000 m. t. d.[7] for the rats.

---

[6] Minimal hæmolytic dose.

Experiments. Rat 1. Toxin 1 c.c. plus 5 % eosin 1 c.c. 1 hour at 37° C in thermostat. Injection of full amount, 10 A.M., Oct. 13. Next day, no tetanus, nor did tetanus develop later. The leg was swollen for several days as result of injection.

Rat 2. Toxin 1 c.c., 5 % eosin 0.5 c.c. Same conditions. Entire quantity injected, Oct. 17. No effect except swelling at injection point.

Rat 3. Toxin 1 c.c., 5 % eosin 0.3 c.c. Same conditions. Injection, Oct. 19, 10 A.M. Oct. 20, slight tetanus; increased next day. Oct. 30, still marked tetanus of injected leg. No general tetanus. Nov. 3, found dead. Lived 15 days.

Rat 4. Toxin 1 c.c., 5 % eosin 0.1 c.c. Same conditions. Oct. 13, 10 A.M., injection. Oct. 14, slight tetanus; next day, marked tetanus. Oct. 19, tetanus increased; dead, Oct. 21. Lived 9 days.

Rat 5. Control. Toxin 1 c.c. Oct. 13, 10 A.M. Next day, marked tetanus. 5 P.M., dead. Lived 29 hours.

These protocols show that eosin in solutions exceeding one per cent. in strength quickly destroys tetanospasmin in the dark, and in 0.6 per cent. solutions reduces greatly the activity of the poison. The chronic nature of the tetanus which the modified poison produces is a striking and interesting occurrence. To what this change is to be attributed—whether to influence of the eosin upon the animal organism or to peculiar changes in the poison itself—has not been determined with accuracy. We shall return to this topic.

### *The Effect in the Animal Body of Eosin upon Tetanus Toxin.*

In this series of tests tetanus toxin and eosin were injected simultaneously but separately into the rat.

Experiments. Rat 1. Oct. 20, 10 A.M. Toxin, 1 c.c. 1 c.c. 5 % eosin immediately injected about the site of introduction of the poison. Oct. 21, slight tetanus, which increased until death on Oct. 24. Lived 4 days.

Rat 2. Same date. Toxin 0.1 c.c. 1 c.c. 5 % eosin immediately injected about toxin site. Oct. 22, no tetanus. Oct. 23, very slight tetanus. Oct. 24, marked tetanus. Increase until death on 7th day.

---

[7] Minimal tetanic dose

Rat 3. Oct. 27, 11 A.M. Injected toxin, 0.05 c.c., and immediately 1 c.c. 5 % eosin. No tetanus, Oct. 30. On 31st, suspicion of tetanus. Next day, tetanus increased, and then progressive increase until death on 8th day.

Rat 4. Oct. 28, 10 A.M. Toxin, 0.01 c.c. and eosin as before. Oct. 30, suspicion of tetanus; 31st, increased slightly; in next days, further increase. Nov. 10, dead. Lived 13 days.

Rat 5. Oct. 29. Toxin, 0.01 c.c., injected into right leg and 5 % eosin solution 1 c.c. injected into left leg. Oct. 30, right leg not used; Oct. 31, marked tetanus. Nov. 3, dead. Lived 5 days.

In this series of experiments the effect of eosin upon the course of the tetanus consisted in delaying the onset of symptoms and in prolonging the period of intoxication. Since the toxin employed caused, in control animals, in doses of 0.0025 cubic centimeter, tetanic symptoms to appear in twenty-four hours, the delay of symptoms in the eosin-treated animals is at once apparent; while the prolongation of life is also well brought out by the protocols. However, none of the animals of this series was actually saved. And in view of this fact the question arises whether, after all, eosin exerts a destructive influence upon the toxin *in vivo* at all comparable with its effect *in vitro*. Since, in all but one rat, the eosin injection was made about that of the toxin, and since eosin alone in five per cent. solution causes œdema of the tissues, perhaps changes in absorption may account for the delay of onset of the symptoms and fatal effect. But this explanation does not entirely account for the effect observed, as in Rat 5 the injection of the poison in one side and the eosin in the other side of the body was made and yet the fatal result was delayed.

## *The Influence of Eosin upon the Course of Tetanus in Rats and Guinea-Pigs.*

It is recognized that poisoning with tetanus toxin is not an exact equivalent of infection with tetanus bacilli, for while in the first the full fatal dose of poison is at once available for fixation by the tissues, in the second the appearance of the first symptoms of tetanus may not exactly coincide with the presence of a fatal quantity of the poison in the body. If, therefore, eosin is capable of modifying tetanus poison in such a manner as to diminish its toxicity when the two are kept in contact for a short time at body temperature, even in diffuse light and in the dark, the important question arises whether this action may be exerted in the body to an extent sufficient to rescue infected animals. To imitate natural conditions more closely silk threads carrying tetanus spores but no toxin were introduced beneath the skin of the right thigh. Some of the animals remained untreated; others were treated with eosin in various ways.

Experiments. Rat 1. Control. Inoculated Nov. 1, 10 A.M. Nov. 4, first appearance of tetanus. Nov. 5, marked tetanus. Dead, Nov. 7. Lived 3 days after the appearance of tetanus.

Rat 2. Control. Nov. 5, inoculation as above. Nov. 8, tetanic. Death, Nov. 11. Survived first appearance of symptoms 3 days.

Rat 3. Nov. 1, 10 A.M. Thread introduced as before. Immediately afterwards 1 c.c. 5 % eosin injected in the tissues above the thread. Nov. 2, œdema; leg not used. Nov. 5, no tetanus; small necrosis at site of injection. Dec. 1, no tetanus; animal normal.

Rat 4. Nov. 1, thread introduced. Nov. 3, first appearance of tetanus. 1 c.c. 5 % eosin injected in same leg. Nov. 4–6, tetanus increased. Nov. 11, dead. Lived 9 days after first appearance of symptoms.

Rat 5. Nov. 1, thread introduced in right leg. Nov. 4, slight tetanus. 1 c.c. 5 % eosin injected in left leg. Tetanus increased. Death on Nov. 8. Survived first symptoms 4 days.

Rat 6. Nov. 1, thread introduced. Nov. 2, no tetanus; 0.5 c.c. 5 % eosin injected about the thread. Nov. 3, no tetanus; eosin injection. Nov. 4, no tetanus; injection repeated. Same on Nov. 5, 6, 7, 8. No further injections. Dec. 3, no tetanus. Animal normal.

Rat 7. Nov. 1, thread introduced. 0.2 c.c. 5 % eosin injected every 24 hours as in Rat 6. Nov. 5, slight tetanus, which gradually progressed until Nov. 12, death. Survived first appearance of symptoms 7 days.

Rat 8. Nov. 1, thread introduced. 0.5 c.c. 5 % eosin injected intraperitoneally every 24 hours for four days. Nov. 5, tetanus appeared; Nov. 12, dead. Survived first symptoms 7 days.

This series of experiments indicates that the power of eosin to destroy or modify the tetanic poison in the body, while marked is not absolute. However the eosin is brought into the body of the infected animals, it causes an increase in the period of intoxication and delay in the supervention of death. If the eosin solution is sufficiently concentrated and is brought into relation with the focus of development of the tetanus spores, it is capable not only of modifying the tetanus but of wholly suppressing its development. The manner of this action is not yet wholly worked out, and hence we cannot at present say whether the suppression affects the germination of the spores or merely the detoxication of the poison as it is produced. This part of the subject, as well as still other phases of it, as, for example, the value of combined eosin and anti-toxin treatment of animals poisoned with tetanus toxin and infected with tetanus spores, the effects of still other photodynamic substances, and the influences of the aniline injections upon local absorption, are now being studied. The influence of eosin upon the production of tetanus toxin *in vitro* is also being determined.

A parallel series of experiments to the latter, carried out on the more highly sensitive guinea-pig, will now be given briefly. The pigs weighed 300 grams each.

Experiments. Guinea-pig 1. Control. Nov. 21, thread introduced. Nov. 22, slight tetanus. Nov. 23, marked tetanus; dead in night. Lived 60 hours.

Guinea-pig 2. Control. Nov. 24, 10 A.M. Tetanus spores. Nov. 25, marked tetanus. Nov. 25, 9 P.M., dead. Survived 36 hours. A third control lived 2½ days.

Guinea-pig 3. Nov. 21, 10 A.M. Spores introduced. 1 c.c. 5 % eosin immediately injected into same leg. Nov. 22, leg swollen; no tetanus. Nov. 23 to 28, no tetanus. Animal emaciating; dead of marasmus, Dec. 1. No symptom of tetanus at any time.

Guinea-pig 4. Nov. 21, 10 A.M. Spores; at same time, 1 c.c. 5 % eosin in opposite leg. Nov. 22, marked tetanus. Died during the night of 23d. Survived 60 hours.

Guinea-pig 5. Nov. 21, 10 A.M. Spores. Nov. 22, slight tetanus. 1 c.c. 5 % eosin injected in locality of thread. Nov. 23, no progress of tetanus. Nov. 24, tetanus increased; no further change until Nov. 27. Animal weaker and tetanus increased. Nov. 28, dead. Survived 6 to 7 days.

Guinea-pig 6. Nov. 21, 11 A.M. Same experiment as No. 5, but the eosin injected into opposite leg Survived 3 days.

Guinea-pig 7. Nov. 21, 10 A.M. Spores. Nov. 22, slight tetanus. 1 c.c. 5 % eosin injected into same leg. Nov. 23, tetanus increased; eosin injection, Nov. 24, eosin injection. Nov. 25, symptoms progressing, eosin injection. Nov. 26, *idem*. Nov. 27, dead. Survived first symptoms 5 days.

This series of experiments shows that even in the highly sensitive guinea-pig tetanic symptoms may be restrained, provided the eosin is brought into close relationship with the developing tetanus poison before it is fixed by the nervous tissues or reaches the general circulation.

# ON THE PHYSIOLOGY OF HEART-BLOCK IN MAMMALS, WITH ESPECIAL REFERENCE TO THE CAUSATION OF STOKES-ADAMS DISEASE.

BY JOSEPH ERLANGER.

(*From the Physiological Laboratory of the Johns Hopkins University.*)

PLATES I.-IV.

## PART II.

## ON THE PHYSIOLOGY OF HEART-BLOCK IN THE DOG.

### INTRODUCTION.

At the present time two views are held with regard to the path taken by the impulse which normally causes the various chambers of the heart to beat. According to the older view, that of the supporters of the neurogenic theory, the impulse arises in the automatic ganglia of the heart and is distributed to the muscle cells through the medium of nerves. According to the more recent view, which is supported by the upholders of the myogenic theory, the impulse arises in the automatically rhythmical musculature of the great veins and is propagated to the various chambers of the heart through their musculature after the nature of a peristalsis.

The literature which has appeared in the support of one or the other of these theories has reached such stupendous proportions that it would be impossible to review it here. An account of it will be found in the recently written reviews of Gaskell,[1] Hofmann,[2] and Langendorff.[3] Here we wish to refer to but one phase of the subject, that which is concerned with the conduction of the impulse from the auricles to the ventricles of the mammalian heart.

---

[1] Gaskell, *Schäfer's Text-book of Physiology*, 1900, ii, 169.

[2] Hofmann, *Schmidt's Jahrbücher*, 1904, cclxxxi, 113.

[3] Langendorff, *Ergebnisse der Physiologie*, 1902, i, pt. ii, 263.

Undoubtedly the greatest stumbling-block for the supporters of the myogenic theory consisted in the view, which was maintained by the anatomists, that in mammals the musculatures of the auricles and ventricles are completely separated from one another by connective tissue. Until it could be shown that this is not the case the myogenic theory could have no sound basis, at least in so far as it concerned the mammals. To His, Jr., belongs the credit of being the first to find a muscular connection,[4] the existence of which means so much to the supporters of the myogenic theory. His has shown, and this has since been abundantly confirmed,[5] that in mammals there is a narrow band of muscular tissue which joins the septum of the auricles with the septum of the ventricles. The anatomy of this bundle, which is rightly termed the auriculo-ventricular bundle of His, has been studied most carefully by Retzer. A free translation of his description of it in man follows. ". . . When viewed from the left side the bundle lies just above the muscular septum of the ventricles and below the membranous septum. In some hearts the muscular septum is so well developed that it envelopes the bundle; it is then difficult to find it. But occasionally it can be seen directly by means of transmitted light. From the left side the bundle can be followed posteriorly no farther than the right fibrous trigone, for here the connective tissue becomes so dense that it is difficult to dissect it away. The impression is therefore received that this mass of connective tissue forms the point of insertion of the bundle. The bundle may be followed anteriorly until it becomes intimately mixed with the musculature of the ventricles."

"When viewed from the right side of the heart the bundle cannot be seen because it is covered by the mesial leaflet of the tricuspid valve whose line of attachment passes obliquely over the membranous septum. To expose the bundle, this leaflet must be cut away from the membranous septum. Then, if the endocardium is removed from the posterior part of the septum of the auricle up to the membranous septum, the posterior part of the auriculo-ventricular bundle will be exposed. If, in addition, the membranous septum be removed, the bundle may be traced from the point to which it could be followed when viewed from the left side as it passes posteriorly over the muscular septum. In the region of the auriculo-ventricular junction it loses its compactness, the fibers divide, and the bundle seems to fork. One branch passes into the superficial part of the valve musculature which descends from the auricles, and the other branch passes directly into the musculature of the auricle."

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[4] His, Jr., *Arbeiten aus der med. Klinik zu Leipzig*, 1903; Ref., Breaunig.

[5] Retzer, *Arch. für Anat.*, 1904, p. 1; Breaunig, *Arch. für Phys.*, 1904, p. 1; Humblet, *Arch. internat. de phys.*, 1904, i, 278.

"Briefly, the auriculo-ventricular bundle runs posteriorly in the septum of the ventricles about 10 mm. below the posterior leaflet of the aortic semilunar valves; with a gentle curve it passes posteriorly just over the upper edge of the muscular septum and sends its fibers into the musculature of the right auricle and of the auricular valves. . . . In the heart of the adult the bundle is 18 mm. long, 2.5 mm. wide, and 1.5 mm. thick."

The anatomy of the auriculo-ventricular bundle in the other mammals is practically the same as in man, but in them its course can be determined only by microscopical examination of sections.

As the auriculo-ventricular bundle of His represents the only demonstrable muscular connection between auricles and ventricles in the heart of mammals, it follows that if the myogenic theory is correct, destruction of this bundle should prevent the passage of the impulse from auricles to ventricles. This is the *sine qua non* of the myogenic theory.

The first attempt to destroy this connection between the auricles and the ventricles in the mammalian heart was made by His, Jr.[6] In this he seems to have succeeded. He states that section of the auriculo-ventricular bundle in the rabbit is followed by complete independence of the rhythms of the auricles and the ventricles. But as he has never made more than a preliminary report of these experiments, and as his tracings have never been published, practically no notice has been taken of his work.

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[6] His, Jr., "Bericht des dritten internationalen Physiologenkongress in Bern," *Zent. für Phys.*, 1895, ix, 469; and *Deutsches Arch. für klin. Med.*, 1899, lxiv, 316.

Very recently, indeed after our experiments had been begun, we came into the possession of an article by Humblet,[7] in which are described some experiments performed with the object of testing the effect of section of the auriculo-ventricular bundle in the heart of the dog. Humblet did not succeed in accomplishing this with the heart *in situ*. He gives but one tracing obtained after section of His's bundle in an excised heart. This tracing shows that complete heart-block resulted.

After a preliminary notice of the experiments herein recorded had been sent abroad for publication there appeared in *Pflüger's Archiv* an article by Hering in which are described the results of cutting the auriculo-ventricular bundle in the perfused heart.[8] Like His and Humblet, Hering found that section of the bundle produced complete heart-block. But Hering carried the research one step further, since he shows that section of the bundle does not interfere with the action of the accelerator nerve on the ventricles.

These are the only researches which bear directly upon this question. Therefore, when our interest in the problem of heart-block was aroused by the studies reported in the first part of this paper, we were impressed with the need of a series of experiments which would put the question of the mode of conduction from auricles to ventricles in the mammalian heart to a thorough test, in the hope that the solution of this problem might throw some light on the pathogenesis of heart-block in man. It was with this object that the experiments to be reported were performed.

## METHODS.

In the first experiments an attempt was made to pass a ligature, carried by a fine curved needle, about the region through which the auriculo-ventricular bundle passes. Of the seven experiments performed, in only one was heart-block obtained.

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[7] Humblet, loc. cit.

[8] Hering, *Pflüger's Archiv*, 1905, lvii, 97.

Examination of the protocols (Exp. 1–7) will show that this was the only experiment (Exp. 3) in which the ligature had been so placed as to include the region of His's bundle.

An attempt was next made to devise a clamp in which the region of the auriculo-ventricular bundle could be compressed just as the auriculo-ventricular junction of the cold-blooded heart may be compressed in Gaskell's clamp.[9] In the first form of clamp devised, the principle of the carpenter's vise was applied. Two bars were each armed at one end with projecting points which were curved in appropriate directions. The points were inserted into the heart so that presumably one lay on each side of the region which contains His's bundle. The bars were so arranged that they could then be conveniently joined together by means of two parallel screws. By driving nuts on these screws the bars could be made to approach one another in parallel, thus compressing the tissue included between them.

Seven experiments were performed with this clamp. In the protocols of these (Exp. 8–14) it may be seen that in two cases (Exp. 8 and 14) heart-block was obtained. These were the only instances in which the clamp had been so placed as to include in its grasp the bundle of His. These results were not considered satisfactory because they could not be nicely controlled and because of the difficulty experienced in placing the clamp properly.

Finally, the following thoroughly satisfactory clamp was devised (Fig. 19.). A fish-hook provided with a very fine barb is bent into the shape of the letter "L." The blunt end of the hook is soldered into the end of a brass bar. That half of the bar to which the hook is attached is square on cross-section and serves as a guide for the brass block, A. The other half of the bar has a fine thread cut in it and carries a nut, B, which may be put in movable connection with the brass block. The edge of the brass block which is directed toward the short arm of the "L" is carefully rounded and is just long enough to fit accurately between the long arm of the "L" and the barb at the end of the hook. The size of the clamp reproduced in Fig. 19 is convenient for dogs.

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[9] Gaskell, *Philosophical Transactions of the Royal Society*, 1882, pt. ii, 994.

To put the clamp on the heart, the heart is first freely exposed by removing the upper two-thirds of the sternum with the adjacent portions of the ribs. The pericardium is opened and stitched to the chest wall so as to lift the heart well toward the surface of the wound. Then, by blunt dissection, the epicardium is split along the line where it passes from the fatty tissue in the auriculo-ventricular groove to the right side of the aorta, a few millimeters posterior to a line which is parallel to the axis of the animal and which passes through the origin of the right coronary artery. Approximately, it is the line which forms the right edge of the mass of fat which covers the anterior aspect of the root of the aorta. By lifting the loose connective tissue away from the wall of the aorta along this line it is possible to arrive at a place immediately above the membranous septum of the ventricles. The point of the hook is set in this place and, while directed obliquely downward and toward the lumen of the aorta, it is thrust into the left ventricle. If the point has been set in the proper place it will be found that it has entered the heart near the line of junction of the right with the posterior leaflet of the aortic valve and usually a few millimeters below the uppermost point of attachment of this valve. The bar is now turned so that the point of the hook is directed caudally, with the short arm of the "L" parallel and close to the ventricular septum. The length of the short arm of the hook has been so chosen that the point will now lie just over the place at which it should be made to enter the ventricular septum. This place is on a perpendicular erected at the middle point of the posterior edge of the membranous septum and a few millimeters removed from this edge. By moving the bar until it lies parallel to the first part of the arch of the aorta, and by pressing the point of the hook in the direction of the ventricular septum, the short arm

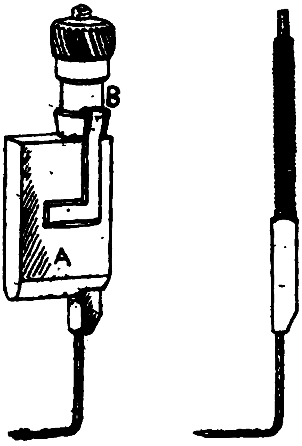

FIG. 19.

of the "L" will perforate the septum at about the right place. The point will enter the right ventricle from 3 to 6 mm. below the auriculo-ventricular junction and somewhat posterior to the anterior edge of the mesial leaflet of the tricuspid valve. The brass block and the nut are then attached to the bar. It might be added that the main trunk of the right coronary artery should not be included in the grasp of the clamp.

This procedure is not difficult: it is only necessary to become familiar with the position which the clamp should occupy, in order to be able to apply it successfully. Since the clamp just described was perfected, heart-block has been produced with it in every one of many experiments.

In the first experiments the contractions of the auricles and ventricles were recorded with the myocardiograph of Roy and Adami. But this instrument occupied so much of the available room that when it was in place great difficulty was experienced in manipulating the clamp. We finally found it most convenient to record the auricular contractions by means of a tambour system. A thread attached to the auricular appendix was made to pull against the head of a receiving tambour. The auricular contractions were therefore recorded as down strokes. A manometer connected with the central end of the carotid artery served to record at one time the movements of the left ventricle through the pulse-waves caused by its contractions, and the arterial pressure. This method has the disadvantage of not showing the exact sequence of auricular and ventricular contractions. The relation which the beginning of the pulse-waves recorded by the manometer bears to the beginning of the ventricular systoles will vary to some extent with the rate of transmission of the pulse-waves and with the time intervening between the beginning of ventricular contraction and the opening of the semilunar valves. As, in this paper, no stress will be laid on small differences in time, these errors will certainly be negligible. For the most part the mercury manometer was used. In a few experiments the Hürthle manometer was used. The time, in fifths of seconds, was recorded on the base line.

All of the animals were anæsthetized with morphine and ether.

## RESULTS.

It is natural that in a research of this kind the same conditions cannot be reproduced with certainty in each experiment. In different experiments the hearts might not have been in the same condition. The clamp might not always have been applied in the same way; it might, for instance, have done permanent injury to the conducting tissue at the moment it was inserted, or it might have been placed in such a position that the conducting tissue was more or less protected from compression. In one experiment the pressure might have been applied more rapidly than in another. Often the same results were not obtained while releasing the pressure on the conducting tissue as had been obtained while compressing the tissue. As a rule, during the return to normal after clamping, more intermediate stages have been obtained than while clamping. This result is to be explained by the fact that the recovery from the injury produced by the clamping is slower than the production of that injury. Naturally, variable results corresponding with these variable and other unaccountable conditions have been obtained in the different experiments. Therefore, in order to present a complete picture of the effect of clamping the region of the auriculo-ventricular bundle, it will be necessary to bring together comparable results of different experiments. Most of these results were obtained while clamping, but a few of them were obtained only while unclamping.

### THE EFFECT ON THE SEQUENCE AND RHYTHM OF THE HEART-BEAT OF GRADUALLY COMPRESSING THE REGION OF THE AURICULO-VENTRICULAR BUNDLE.

If the clamp has been properly adjusted on the heart, the heart will continue to beat with undisturbed sequence and rhythm. Its rate may be somewhat increased. Upon cautiously tightening the clamp, the first obvious effect may be an increase in the period intervening between the beginning of an auricular contraction and the beginning of the ventricular contraction, *the intersystolic period*, of the same cardiac cycle. The duration of the intersystolic period may then gradually increase in the successive cycles until eventually the ventricles fail to respond to one of the auricular contractions (Fig. 20, G). Immediately following this ventricular "silence," the intersystolic period again becomes short and it again increases, at first slowly, then rapidly just before the next ventricular silence. At first the ventricular beats may be dropped rather irregularly: the longest interval between two ventricular silences has been twenty-seven beats (Fig. 20, G). But soon the ventricular silences recur regularly: the same small number of normal beats may always be interposed between them. Just before each ventricular silence the intersystolic period lengthens quite rapidly. Stages have been obtained which show one ventricular silence recurring regularly in ten, nine, eight, seven, six, five, four, three, and two auricular beats. (Fig. 20; F, E, D, C, B, A.)

Many of the results above referred to were obtained in an experiment in which the Hürthle manometer was used to record the arterial pressure on a rapidly moving surface (Exp. 27). The results obtained in this experiment indicate that the conducting tissue had not been nicely included in the clamp, or that it had been protected by some other structure. This is borne out by the fact that to obtain the different stages of block it was found necessary in this experiment to tighten the clamp through a considerable distance, and that the last stage usually obtained while clamping was attained with great difficulty. These results are in sharp contrast with those of Exp. 31.

But usually upon clamping the normal sequence will more or less abruptly give way to a partial block in which the ventricles respond only to every other beat of the auricles, giving a 2:1 rhythm (Fig. 21). Frequently before the final change occurs there may be some irregular alternation between the normal sequence and the 2:1 rhythm. With further clamping a 3:1 rhythm may be established, preceded, usually, by some preliminary alternation between a 2 : 1 and a 3 : 1 rhythm. This will then give way to complete block: the beats of the ventricles will be totally independent of those of the auricles and very much slower than them. The inverse order of changes in rhythm may be obtained when the clamp is unscrewed, provided care has been taken to prevent permanent injury of the tissue included in the clamp. These changes in the conductivity at the auriculo-ventricular junction of the dog's heart may be obtained as regularly and as promptly as in experiments in which the auriculo-ventricular junction of the cold-blooded heart is compressed in Gaskell's clamp. The stages between normal sequence and complete block cannot, as a rule, be maintained over a long period of time. In the course of time, even though the amount of pressure exerted by the clamp remain the same, a partial block will pass into complete block or into the normal state, according as the clamp had previously been tightened or loosened. But it is possible to keep the block complete for over an hour and still have the normal sequence return promptly upon loosening the clamp (Exp. 15).

The picture presented by the exposed heart with complete block at the auriculo-ventricular junction may readily be formed. But from the standpoint of the diagnosis of the condition in the human being it is important to know exactly what may be seen. The ventricles, of course, beat much more infrequently than the auricles. After the ventricles have emptied themselves, it may be seen that each contraction of the auricles sends into the former a distinct wave, upon the subsidence of which the volume of the ventricles is seen to have been considerably increased. In this way the ventricles are rhythmically distended by the contractions of the auricles. Likewise, it may be seen that each contraction of the auricles sends a wave into the great veins. After two or three such waves have occurred the ventricles contract, but this contraction bears no constant time relation to the contractions of the auricles. These two perfect and independent rhythms which are characteristic of complete block may therefore be seen upon inspection of the great veins or of the ventricles. A cardiogram obtained by resting the receiving tambour directly upon the ventricles of the heart while in complete block is reproduced in Fig. 22. In it the beats of the auricles and of the ventricles may be distinctly seen. This tracing should be compared with Figs. 1 and 2 (Part I), in which are reproduced comparable tracings obtained from man.

The rate of the ventricles when beating independently of the auricles has varied within rather wide limits. As is shown in Table XVI, at the beginning of an experiment it has varied between 34.8 and 69.8, the average being 54.1. Toward the close of the experiments it has been somewhat slower. No attempt has been made to determine the cause of this variability, but the temperature of the exposed heart is probably one of the most important factors. As may be seen in the table, the inherent rate of the ventricles has always been considerably less than that of the auricles, the average ratio, $\frac{A}{V}$, being 3.05. (Compare with values obtaining in man.)

TABLE XVI.

SHOWING THE AURICULAR AND VENTRICULAR RATES OF THE DOG'S HEART DURING COMPLETE BLOCK AT THE BEGINNING OF EXPERIMENTS, INCLUDING, FOR COMPARISON, A FEW ESTIMATIONS IN MAN.

| No. of Exp. or Case. | No. of Vs. | Duration in ⅕ Sec. | V. Rate per Min. | No. of As. | Duration in ⅕ Sec. | A. Rate per Min. | $\frac{A}{V}$ | Max. Pres. | Min. Pres. | Remarks. |
|---|---|---|---|---|---|---|---|---|---|---|
| 14 | 8 | 69.0 | 34.8 | 32.0 | 74.0 | 130.0 | 3.7 | 41 | 21.5 | |
| 15 | 20 | 86.0 | 69.8 | 62.0 | 86.0 | 216.0 | 3.1 | 59 | 49 | |
| 18 | 8 | 43.0 | 55.8 | 25.3 | 43.0 | 177.0 | 3.2 | 79 | 58 | |
| 19 | 16 | 70.6 | 68.0 | 37.7 | 70.6 | 160.2 | 2.4 | 76 | 70 | |
| 20 | 8 | 50.0 | 47.8 | 26.4 | 50.2 | 157.7 | 3.3 | 94 | 88 | |
| 21 | 8 | 53.5 | 44.9 | 21.0 | 53.5 | 117.8 | 2.6 | 116 | 98 | |
| 24 | 7 | 36.3 | 57.8 | 21.3 | 36.3 | 175.8 | 3.04 | — | — | |
| Average. | | | 54.1 | | | 162.1 | 3.05 | | | |
| Case I. | | | 22.4 | | | 79.6 | 3.55 | | | Extremes under normal conditions |
| " " | | | 31.0 | | | 84.6 | 2.73 | | | |
| " II. | | | 31.7 | | | 89.3 | 2.82 | | | Only determination. |

## THE EFFECT ON THE BLOOD-PRESSURE OF VARYING THE SEQUENCE OF THE HEART-BEAT.

With each change in rhythm the blood-pressure is materially affected. (See protocols in the Appendix.) It falls whenever the rate of the ventricles is decreased and rises whenever the rate of the ventricles is increased. The results obtained in the different experiments vary considerably among themselves. It will, however, be seen that a fair pressure may be maintained even in complete block. (See Fig. 21.)

Unfortunately, no systematic study was made of the variations of the systolic and diastolic pressures which are associated with the various changes in the auriculo-ventricular rhythm. However, a clue to the nature of these pressure changes may be obtained from examination of the blood-pressure record made with the Hürthle manometer (Fig. 20). It may be seen that in the occasional ventricular silences the systolic pressure is not materially affected, whereas the diastolic pressure falls decidedly. (Compare with page 722 of Part I.)

## THE INFLUENCE OF THE EXTRINSIC CARDIAC NERVES ON THE AURICLES AND VENTRICLES IN VARIOUS STAGES OF HEART-BLOCK.

*Vagus Effects.*—In any stage of partial block the auricles and the ventricles may be completely inhibited by stimulation of the vagus nerve: inhibition is obtained at least as easily as under normal conditions. (Fig. 23, A and B). But when the block is complete, stimulation of the peripheral end of the vagus (Figs. 23, C, and 24), although it inhibits the auricles, usually has little effect upon the rate of the ventricles. Indeed, in one experiment (Exp. 8) the rate of the ventricles increased during vagus stimulation. Occasionally a slight diminution in the ventricular rate results. Furthermore, when the heart-block is complete, stimulation of the vagus nerve has but little or no influence upon the force of the ventricular beats, as judged of by variations in the arterial pressure. Thus, during stimulation of the vagi, the arterial pressure may not be affected at all or it may fall slightly.

How much of this decrease of the arterial pressure is to be attributed to the falling away of the filling effect of the auricular contractions, and how much to a vagus effect upon the force of the ventricular contractions, cannot be determined. But it is certain that the former factor is not unimportant. Thus it may be seen in Fig. 25 that the ventricular contraction which follows immediately upon an escape beat of the auricles produces a very much larger pulse-pressure than the other ventricular contractions.

The loss of the influence of the vagi over the ventricles is always exactly synchronous with the establishment of complete heart-block. This association is so constant that the reaction of the heart to stimulation of the vagus nerve may be used as a test to determine the presence or absence of complete block.[10]

In two experiments (Exp. 19 and 30) a 2 : 1 rhythm has temporarily changed into a 1 : 1 rhythm during recovery from vagus inhibition. This change was in all probability associated with a slowing of the auricular rate; although it may possibly have resulted from an increase in the conductivity of the heart muscle which, according to Gaskell,[11] may follow vagus stimulation in the tortoise. Stimulation of the vagus nerve during partial heart-block has never increased the resistance to the passage of impulses between auricles and ventricles—at least it has never caused a change in the direction of complete block.

When the block is complete, section of both vagi has no, or but a very slight, effect upon the rate of the ventricles, although the usual change in the rate of the auricles may occur (Exp. 15). In one experiment (Exp. 19), after having cut one vagus, freezing and thawing of the intact vagus nerve had no effect upon the rate of the ventricles. It should, however, be stated that in all of these experiments, excepting Exp. 15, the tone of the vagus center was nil, if we may use as an index of the degree of tone the effect of section of both vagi on the rate of the auricles. In the single exception, section of both vagi increased the rate of the auricles from 213 to 245 beats per minute. The ventricular rate increased but 2.1 beats per minute—from 46 to 48.1.

With but a single exception, section of both vagi has had no effect upon the force of the ventricular contractions as judged of by the effect on the blood-pressure. In the single exception, which has been mentioned above, section of both vagi was followed by a marked and prolonged rise of the blood-pressure. We have not been able to duplicate this result.

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[10] Krehl and Romberg (*Schmiedeberg's Archiv*, 1892, xxx, 49) have noted the same association in cases where the block was produced by crushing all of the tissue in the auriculo-ventricular junction. We have since met with exceptions to this rule in 3 : 1 rhythms (Erlanger and Hirschfelder, *Amer. Jour. of Physiol.*, Jan., 1906.)

[11] Gaskell, *Jour. of Phys.*, 1883, iv, 96.

*Accelerator Effects.*—When the heart-block is complete, stimulation of the accelerator nerve, as a rule, increases the rate of the ventricles as well as that of the auricles[12] (Exp. 20 and 21). The increase in the rate of the ventricles may be as great proportionally as that of the auricles. Indeed, in some tests (see Exp. 20 and 21) stimulation of the accelerator nerve has caused a decided increase in the rate of the ventricles and no change, or even a slight decrease, in the rate of the auricles. These last results were usually obtained when strong stimuli were employed, and it seems probable that the anomaly was caused by an escape of the stimulus, resulting in either direct or reflex inhibition of the auricles. This association therefore serves as a beautiful illustration of the fact that in complete heart-block the ventricles are but little affected by impulses arriving in the heart through the vagi. But the fact that the auricles are affected by the escape of stimulus, whereas the ventricles are not so affected, prevents the formulation of a positive conclusion as to the relative effect on the auricles and ventricles of stimulation of the accelerator nerve.

## THE INFLUENCE OF OTHER CONDITIONS ON THE RATE OF THE VENTRICLES IN COMPLETE HEART-BLOCK.

The rate of the ventricles when beating independently of the auricles is not materially affected by variations of the blood-pressure produced by compression of the abdominal or thoracic aorta (Exp. 14 and 15), nor by extreme hæmorrhage (Exp. 21).

In one experiment (Exp. 15) the effect was tested of cutting both splanchnics. But this test was made near the close of a prolonged experiment, when the blood-pressure was low, and the heart-rate very slow—probably a temperature effect. The results of this test are, therefore, of but little value.

[12] Hering (loc. cit.) has recently noted that stimulation of the accelerator nerve accelerates the rate of the ventricles when they are beating independently of the auricles.

General asphyxia carried to the convulsive stage does not materially affect the rate of the ventricles in complete block (Exp. 15 and 18). If there is any change, it is in the direction of an increase in rate.

The blocking of one coronary artery, either the right or the left, by Porter's method [13] (Exp. 19 and 20) has not obviously altered the rate of the independent ventricles. In one experiment (Exp. 20) no marked change in the rate of the ventricles occurred after the left coronary had been plugged for four minutes. The ventricles will continue to beat for many minutes after excision of the heart. It is possible that these results are to be accounted for by a low temperature of the heart from prolonged exposure, rather than by an increase in the resistance of the ventricles when they are no longer controlled in the normal way.

## UNUSUAL SLOWING OF THE VENTRICULAR RATE UPON CLAMPING.

In many experiments (Exp. 21, 22, 23, 26, 30, and 31) while carefully tightening the clamp the ventricular rate alone has abruptly become remarkably slow (see Fig. 26). As a rule, the slowing of the ventricular rate is most marked at the very onset of this phenomenon. The interval between two ventricular contractions has upon several occasions been as long as twenty seconds. The ventricular rate then gradually increases until a rate obtains which is usual for heart-block. The auricular rate does not seem to be affected. The blood-pressure may fall almost or quite to zero.

This condition is a most remarkable one to witness. Usually while carefully tightening the clamp the ventricles stop beating without warning. The auricles continue to beat with an apparently unaltered rate. With each contraction of the auricles there is a sudden, slight increase in the volume of the ventricles. The ventricles thus enlarge with each contraction of the auricles until their distension becomes really huge. The veins, too, are distended and pulsate. Respiratory convulsions may begin.

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[13] Porter, *Jour. of Exper. Med.*, 1896, i, 60.

Witnesses are almost convinced that the experiment has come to a close when, it may be after more than twenty seconds, the ventricles suddenly empty themselves with one great effort. Then there succeeds another long interval during which the ventricles remain perfectly flaccid and are gradually distended by the contractions of the auricles. But usually this pause is not quite so long as the first one, and the succeeding periods become shorter and shorter until the state usually seen in heart-block is established. Although the condition appears to be extremely grave, death has never resulted from it.

In almost every instance in which marked slowing of the ventricular rate occurred it was not preceded by any of the stages of heart-block, although the usual care in tightening the clamp was, as a rule, observed. Occasionally it was preceded by a few 2 : 1 or 3 : 1 cycles. On the other hand, instances were observed in which heart-block developed just as abruptly, but in which the change was not preceded by this remarkable slowing of the ventricles (Exp. 28). In a few experiments (Exp. 31) the maximum slowing of the ventricles did not come on at once. In these the maximum slowing was reached through one or two ventricular cycles of increasing duration. Very rarely in instances in which the block was slowly established, the ventricular rate was somewhat slower at the moment of onset of complete block than a little later.

In every instance in which this phenomenon of marked preliminary ventricular slowing was observed, complete heart-block was found to exist when the ventricular rate became constant. Upon releasing the clamp after the conditions have become constant, the recovery to normal usually occurs through short stages of 3 : 1 and 2 : 1 rhythms, provided the conducting structures have not been severely injured.

At post-mortem examinations of these cases in which marked slowing of the ventricular rate has occurred, the impression is usually received that the auriculo-ventricular bundle has been unusually favorably exposed to the action of the clamp. We are, however, not at all certain as to the cause of this phenomenon, and therefore a discussion of the matter will be deferred until a special investigation of it has been made. However, the protocols of a few experiments that have thus far been performed in the effort to throw light upon this subject will be found in the Appendix[14] (Exp. 22, 23, 24, 25, 26, 27, 30, and 31).

## THE POSITION OCCUPIED BY THE CLAMP IN SUCCESSFUL AND UNSUCCESSFUL EXPERIMENTS.

In every experiment in which heart-block has been obtained it has been found at autopsy that the clamp had undoubtedly embraced the region in which the auriculo-ventricular bundle lies. But in order to have more exact control of this most important condition, in a few promiscuously chosen instances (Exp. 17, 18, 19, and 21) the tissue included in the clamp has been examined microscopically for the presence in it of the auriculo-ventricular bundle of His. These histological examinations, which have been made by Retzer,[15] have invariably shown that the auriculo-ventricular bundle had been included in the clamp.

In most of the early experiments, heart-block was not obtained upon tightening the clamp or ligature. And in some of the recent experiments heart-block was not obtained at the first attempt. In these instances, the hook was withdrawn from the muscular septum and reinserted until a position was obtained at which tightening of the clamp produced heart-block. At the post-mortem examinations in these experiments the earlier positions that the clamp had occupied were sometimes marked by hæmorrhages into the tissue where it had been perforated by the point of the hook and where the tissue had been compressed in the effort to block the passage of the impulse. In all of these unsuccessful instances, the position which the clamp or ligature had occupied indicated that the auriculo-ventricular bundle had not been included in their grasp or had probably escaped compression for some other reason (Exp. 16).

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[14] Since this paper was forwarded for publication this investigation has been completed and the results of it will appear in the *American Journal of Physiology*, 1906, No. 1.

[15] I wish here to express my thanks to Dr. Retzer for his kindness in making these valued and time-consuming examinations for me.

We have performed no experiments to determine the effect of destroying all connection between the auricles and ventricles excepting the region of the auriculo-ventricular bundle. This check was considered unnecessary because it has been so frequently made by other investigators. Krehl and Romberg investigated this problem directly, and as a consequence of *negative* results they have concluded that the impulse which normally causes the ventricles to contract passes through the auriculo-ventricular bundle of His. More recently Hering [16] has cut all of the auricular tissue, excepting the septum, away from the ventricles in the artificially perfused heart. No disturbance in sequence resulted. Furthermore, both Wooldridge [17] and Tigerstedt [18] state that in order to obtain heart-block it is necessary to destroy completely the functional continuity between auricles and ventricles. It can be easily understood that in experiments like those of Wooldridge and of Tigerstedt, in which the auriculo-ventricular junction is tied off *en masse*, or in which an attempt is made actually to cut across this region, the auriculo-ventricular bundle, owing to its protected position, would be apt to escape destruction. The evidence is therefore complete that the impulse which normally causes the ventricles to contract passes through the region of the auriculo-ventricular bundle.[19]

[16] Hering, *Zent. für Phys.*, 1903, xvii, 1.

[17] Wooldridge, *Arch. für Phys.*, 1883, 522.

[18] Tigerstedt, *Arch. für Phys.*, 1884, 497.

[19] The observations of Lomakina (*Zeit. für Biol.*, 1900, xxxix, 385) alone differ from those of other investigators. These are some of her conclusions: "Isolirte Unterbindungen oder Durchhitzungen solchen [Verbindungs] Fasern [nerves] stören die coordination zwischen Vorhöfen und Kammern, oder zwischen den beiden Kammern." "Nicht nur trennende Ligaturen zwischen Vorhöfen und Kammern unterbrechen den functioneller Zusammenhang, sondern auch Unterbindungen von oberen Theilen der Vörhofe und sogar besonders Schnurungen um die grossen Herzgefässe oberhalb des Herzens."

The marked discrepancies between these results and those of all other investigators who have studied this problem are perfectly obvious. Furthermore, the results obtained by Lomakina in presumably the same procedure differ so widely in the different experiments that we feel certain that faulty technique alone will account for them.

### ADDENDUM.

In an experiment which has recently been performed by the author in collaboration with A. D. Hirschfelder, a 1 : 1 rhythm was changed into a 2 : 1

## THEORETICAL CONSIDERATIONS.

*Is the Functional Connection between Auricles and Ventricles of the Mammalian Heart Muscular or Nervous?*—Histological investigations have shown that the only demonstrable muscular connection between the auricles and ventricles of the mammalian heart consists of the auriculo-ventricular bundle of His. The experiments herein reported, as well as those of others, have shown conclusively that damage to the tissue in the region in which this bundle lies alone will obstruct the passage of impulses from auricles to ventricles. These facts certainly afford logical evidence that in the case of the mammalian heart the impulse which normally causes the ventricles to contract is conducted through muscular tissue. In order to make this evidence absolutely conclusive it would be necessary to show that the tissue which has been included in the grasp of the clamp in successful experiments contains no nerve fibers. Naturally, this is not the case. There will undoubtedly be found in this tissue the nerve fibers which have been seen to ramify over the cells of heart muscle everywhere,[20] and nerve fibers which lie under the epicardium. But it certainly is improbable that such an important function as the conduction of the normal cardiac impulse is performed by scattered nerve fibers located in a limited region. If, as has been shown, the function of conduction is localized in a very limited region it seems reasonable to demand that a definite structure, whose function it is to conduct, be found in this region. If the conduction is through nerve there should

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rhythm by pouring warm salt solution over the exposed auricles; and the 2 : 1 was converted into a 1 : 1 rhythm by pouring cold salt solution over the auricles. In the former procedure the auricular rate was increased, in the latter it was diminished. In addition we have shown that the phenomenon of stoppage of the ventricles may be obtained after the rhythm has been 2 : 1 for a long period of time, and that stoppage may occur in the fully atropinized heart. And finally, in this experiment the animal would almost certainly have died as a result of stoppage of the ventricles had not the ventricles been made to beat by artificial stimulation.

[20] See Berkley, *Johns Hopkins Hospital Reports*, 1895, iv, 748. Nerve fibers ramifying through the auriculo-ventricular bundle have since been found by Tawara, *Zent. für Phys.*, 1905, xix, 298.

be found in the region through which the impulse is conducted a definite nerve trunk or a well-developed nerve plexus. Retzer has made a careful examination of serial sections of the tissue included in the clamp in four successful experiments (Exp. 17, 18, 19, and 21) of this research. He informs me that the tissue contained no nerve trunks. And His states that no nerves are to be found in this region.[21] Therefore we maintain with Hering, that until it can be shown that the impulse which normally causes the ventricles to contract is conducted through the scattered nerve fibers which lie in the region of the auriculo-ventricular bundle, it must be admitted that the function of conduction between auricles and ventricles of the heart of the dog is performed by the muscular tissue of the auriculo-ventricular bundle.

This mode of conduction of the cardiac impulse may not be the same in all species of animals. It is undoubtedly the same in the case of the rabbit[22] and of man as it is in the dog. But Carlson[23] has obtained what appears to be convincing evidence that in the heart of limulus the conduction is through nerve. We do not, however, believe that Carlson is justified in inferring from his experiments that conduction in the vertebrate heart is the same as in the crustacean heart. To justify such an assumption it must first be shown that the physiological properties of the heart muscle of limulus are the same as those of the heart muscle of mammals. If we may be justified in assuming that the physiological properties of the heart muscle of the American lobster are the same as those of the heart muscle of limulus, then it was first shown by Hunt, Bookman, and Tierney[24] that the crustacean heart muscle has none of the characteristic properties of vertebrate heart muscle excepting, perhaps, very limited spontaneous rhythmicity. Indeed, in its physiological characteristics, the heart muscle of crustaceans resembles skeletal muscle much more closely than it does vertebrate heart muscle. Thus it has a very short latent period, it has no refractory period, its contractions may be summated and therefore tetanus may be obtained, and it does not obey the "all or none" law. In part, the observations of Hunt and his co-workers have been confirmed by Carlson[25] in the case of limulus. But Carlson seems to think it unnecessary to bring these facts into connection with the curious reactions of the heart of limulus to stimulation of extrinsic nerves. To one who has never worked with the invertebrate heart it does not seem at all surprising to find that a heart whose muscle has most of the properties of skeletal muscle, is governed more after the fashion of such a muscle than of the heart muscle of vertebrates.[26]

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[21] "Nerven verlaufen in diesem Bündel nicht."

[22] His, loc. cit.

[23] Carlson, *American Jour. of Phys.*, 1905, xiii, 217. Here references to earlier papers by the same author will be found.

[24] Hunt, Bookman, and Tierney, *Zent. für Phys.*, 1897, xi, 274.

*On the Cause of the Intersystolic Pause.*—Hering[27] has suggested that the presence in the mammalian heart of a long, narrow, muscular bundle through which the impulse must pass on its way from the auricles to the ventricles explains very satisfactorily the delay between the beginning of auricular systole and of ventricular systole. This delay, which is so important to the proper functioning of the heart, was explained by Gaskell[28] in a similar way for the cold-blooded heart. Gaskell showed that to produce a block to the passage of the impulse in the cold-blooded heart it is only necessary to narrow down, by section, the muscle connecting the arterial with the venous ends of the heart. Furthermore, he found in the auriculo-ventricular junction of the tortoise heart circular strands of muscle more embryonic in structure than the adjacent musculature. These, he believes, conduct the impulse from auricles to ventricles. The slowness of the passage of the impulse he believes is due in part to the embryonic structure of the connection and in part to the indirectness of its course. It is interesting to note that these observations of Gaskell can be applied without any essential modification to the conditions that obtain in the mammalian heart. For the auriculo-ventricular bundle of His lies very largely in the plane of the auriculo-ventricular junction and, according to Retzer,[29] its fibers have not the same staining properties as have those of the rest of the heart muscle.

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[25] Carlson, *American Jour. of Phys.*, 1905, xii, 471.

[26] See Howell, *Text-book of Physiology*, 1905, 498.

[27] Hering, *Pflüger's Archiv*, 1905, lvii, 104.

[28] Gaskell, *Jour. of Phys.*, 1883, iv, 70.

In the case of the mammalian heart it cannot be proved that the length of the intersystolic pause depends upon the length and physiological properties of the auriculo-ventricular bundle. But it can and has been shown that slight compression of this bundle will distinctly delay the passage of the impulse from auricles to ventricles. The methods employed for recording the movements of the auricles and ventricles in this research were not sufficiently accurate to permit of exact measurement of the length of this delay. But they were sufficiently accurate to warrant the statement that the change in the rate of conduction which results when His's bundle is compressed is much greater than could possibly occur if the impulse were conducted through nerve fibers.[30]

*The Action of the Vagi on the Ventricles.*—One of the most constant results obtained while clamping the auriculo-ventricular bundle is the simultaneous appearance of complete heart-block and the complete, or almost complete, disappearance of the vagus control over the ventricles. This result may be interpreted in two ways:

(1) The vagi do not act directly upon the ventricles: stoppage of the ventricles when the vagus is stimulated is a consequence of the stoppage of the auricles, i. e. (and this amounts to the same thing), it is the consequence of the falling out of stimuli which normally cause the ventricles to contract.

(2) The vagi exert a positive influence upon the ventricles through the medium of the auriculo-ventricular bundle; clamping this bundle blocks the passage of this influence.

No conclusive evidence in the support of one or the other of these ideas has been obtained. But the facts (*a*) that the ventricles usually continue to beat with their normal inherent rhythm at the moment complete heart-block is established, whereas (*b*) they stop beating when the auricles are inhibited, indicate that stoppage of the ventricles when the vagus is stimulated is the result of the arrival in the ventricles of a positive influence during inhibition.[31]

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[29] Retzer, verbal communication. It has since been found by Tawara (l. c.) that the fibers of the auriculo-ventricular bundle belong to the Purkinje system.

[30] We know of no research in which the effect of compression on the rate of transmission of the nervous impulse has been studied. Our statement is based upon the effect on nerve of other insults, for example, cold (Helmholtz, *Müller's Archiv*, 1850, 276).

It might in addition be urged that when the block is complete the ventricles beat so slowly that, to use the suggestion of Gaskell,[32] anabolism is always at its maximum. Therefore stimulation of the vagus will produce no further anabolism and consequently the ventricles cannot be stopped. But the facts do not support this idea. When the rhythm is 2 : 1 or 3 : 1, the ventricles may be inhibited in most instances as readily as when the rhythm is normal. When the block is complete the rate of the ventricles may not be much slower than when the rhythm was 2 : 1 or 3 : 1, but now the ventricles cannot be inhibited by stimulation of the vagus.

But it will be recalled that, at the moment complete block is established, a very marked slowing of the ventricular rate may occur. If this marked slowing of the ventricular rate is the result of the sudden withdrawal of the stimuli of auricular origin, then it might be possible to ascribe stoppage of the ventricles when the vagi are stimulated to an absence of stimulation. The fact that stoppage of the ventricles does not always occur when the block becomes complete does not constitute an insuperable obstacle to the acceptance of this idea. For it is possible that when the block is slowly established time is allowed for something to occur which permits the ventricles to assume their maximum inherent rate at once. But, on the other hand, in some experiments in which complete block has been suddenly established there was no marked preliminary slowing of the ventricular rate. Still it is possible that the great variability in the reaction of hearts to stimulation of the vagus may account for such exceptions.

[31] Tigerstedt, *Physiologie des Kreislaufes*, 1893, 250.

[32] Gaskell, *Philosophical Transactions of the Royal Society of London*, 1882, pt. ii, 1029.

The fact that during complete block stimulation of the vagus may sometimes slow the ventricular rate very slightly and cause a slight fall of the blood-pressure may be interpreted in two ways. (1) A block which will effectually prevent the passage of the normal stimulus may not effectually prevent the passage of an inhibitory influence. (2) Inhibitory influences may reach the ventricles by other ways than through the auriculo-ventricular bundle. The vagus effect which has been observed during complete block has, however, been so very slight that it can be of but very little significance.[33]

It will therefore be seen that the problem of the mode of action of the vagi upon the ventricles is intimately associated with the phenomena upon which is dependent the stoppage of the ventricles occurring at the moment when complete block is established. Although some effort has been made to solve the latter problem, a sufficient number of facts has not yet been collected to justify a final statement with regard to it.

### CONCLUSIONS OF PART II.

The more important results of the second part of this paper may be summarized as follows:

(1) In the dog the impulse which normally causes the ventricles to contract is conducted through the auriculo-ventricular bundle of His.

(2) By compression of this bundle all stages of heart-block may be obtained. These include:

- (*a*) An increase of the intersystolic pause;
- (*b*) An occasional ventricular silence;
- (*c*) Regularly recurring ventricular silences—e.g., one silence in ten, nine, eight, seven, six, five, four, three, and two auricular beats;
- (*d*) A 2 : 1 rhythm;
- (*e*) A 3 : 1 rhythm;
- (*f*) Complete heart-block.

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[33] It is indeed possible that these effects may be wholly or in part due to the inhibition of the auricles.

(3) As a rule the ventricles take on a constant slow rate at the moment complete heart-block is established. Occasionally, usually when the block becomes complete suddenly, there results a marked preliminary slowing of the ventricular rate. The auricular rate does not change.

(4) When the block is complete, stimulation of the vagus nerve has no, or but a minimal, effect upon the rate and force of the ventricular beats, whereas the auricles still react normally.

(5) When the block is complete, stimulation of the accelerator nerve increases the rate both of the auricles and the ventricles.

(6) When the block is complete, the rate of ventricular beats may not be materially affected by variations in the general blood-pressure, nor by asphyxia, nor by interference with the coronary circulation.

## PART III.

## THE RELATION OF HEART-BLOCK TO STOKES-ADAMS DISEASE.

### THE ANALOGIES BETWEEN HEART-BLOCK IN MAN AND HEART-BLOCK IN THE DOG.

A mere perusal of Parts I and II of this paper should suffice to impress upon the reader the close relation which exists between the results obtained from a study of a case of heart-block in man and heart-block in the dog. These relations are so close that there is no reason to doubt that the condition in man is caused by the same factor which produces the condition in the dog. That heart-block in man is the result of a diminution in the conductivity of the auriculo-ventricular bundle of His, is borne out not alone by the fact that a condition of heart-block exists, but also by the facts that the vagus has lost its influence over the ventricles, that presumably the accelerator nerves still preserve their influence over the ventricles, and that the ventricles may stop beating for long periods of time while the auricles continue to beat without interruption.

It is true that in man marked slowing of the ventricular rate usually occurs at times when the block is complete, whereas in the dog we have not been able to obtain a marked reduction of the ventricular rate after complete heart-block has been established. But we believe that this difficulty is merely the result of the crudeness of experimental methods as compared with the subtle inroads of pathological processes. For example, in animal experiments it is not possible to maintain a partial block for any considerable length of time. Usually within a very few minutes the partial block will disappear either in one direction or the other. But in man the condition of partial block may be maintained for days at a time. And when it is recalled that the cause of the slowing of the ventricles both in man and in the dog is obscure, the mere fact that in both marked slowing of the ventricles alone may occur should suffice to establish the identity of the lesion. We therefore state without hesitation that in our case of Stokes-Adams disease the lesion upon which the symptoms depended involved the auriculo-ventricular bundle of His.

## WILL A LESION IN THE VICINITY OF THE AURICULO-VENTRICULAR BUNDLE ACCOUNT FOR ALL OF THE TYPICAL SYMPTOMS OF STOKES-ADAMS DISEASE ?

The cardinal symptoms of Stokes-Adams disease, all of which were noted by Stokes,[34] are:

(1) A slow pulse sometimes associated with pulsations in the veins of the neck which may be more than twice as frequent as the manifest beats of the ventricles.[35]

(2) Syncopal attacks which may be epileptiform or apoplectiform in character and in which the pulse-rate is unusually slow.

With regard to the former symptom, it is perfectly obvious from what has already been said that in heart-block the pulse is slow and that the auricles will usually develop venous pulsations of more than twice the frequency of the apex beats.

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[34] Stokes, *Dublin Quart. Jour. of Med. Sciences*, 1846, ii, 73.

[35] Stokes makes the significant statement that "the number of reflex pulsations (in the veins) is difficult to be established, but they are more than double the number of the manifest ventricular contractions." As this statement precedes the publication of the epoch-making experiments of Stannius, it does not seem probable that Stokes meant to imply that the auricles and ventricles were beating independently of one another.

And with regard to the latter, it has also been shown that in heart-block there may be periods during which the pulse-rate is unusually slow even for heart-block. Evidence has also been presented that the syncopal attacks are certainly the result of slowing of the ventricular rate. And Webster[36] first forcibly called attention to the fact that the epileptiform seizures of Stokes-Adams disease may be caused by the anæmia of the brain resulting from failure of the heart to supply a sufficient quantity of blood. For Kussmaul and Tenner,[37] and many others since them, have shown that "the convulsions appearing in profuse hæmorrhage of warm-blooded animals (including man) resemble those observed in epilepsy," and that "when the brain is suddenly deprived of its red blood, convulsions ensue of the same description as those occurring subsequent to ligature of the great arteries of the neck."

The apoplectiform attacks might also be accounted for in much the same way. Kussmaul and Tenner have shown that "venous congestion of the brain, as well as arterio-venous congestion, brings about conditions which belong more to those of apoplexy than to those of epilepsy, and are characterized by paralysis of the glottis, together with a slower respiration and slight spasmodic symptoms." This state of affairs might obtain in those syncopal attacks in which the slowing of the ventricles is not sufficient to cause convulsions, but will just cause complete unconsciousness. In such a case the venous congestion would be marked, and the fall of the arterial pressure might not be extreme.

It is therefore evident that all of the cardinal symptoms of Stokes-Adams disease may be duplicated by heart-block and its attendant phenomena.

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[36] Webster, *Glasgow Hospital Reports*, 1900, iii, 413. See also, Bristowe, *Lancet*, 1885, i, 447; and 1894, ii, 671; and Gibbings, *Lancet*, 1885, i, 288.

[37] Kussmaul and Tenner, *Epileptiform Convulsions from Hæmorrhage*, New Sydenham Society, 1859.

## IS HEART-BLOCK ALWAYS THE CAUSE OF STOKES-ADAMS DISEASE?

It has been shown that all of the symptoms of Stokes-Adams disease may be caused by a lesion which involves either directly or indirectly the auriculo-ventricular bundle of His—i. e., by heart-block. It has also been shown that in the two typical cases of Stokes-Adams disease herein described heart-block did exist. The question then arises, Is heart-block the pathogenetic factor of all cases of Stokes-Adams disease?

With the object of answering this question it was at first intended to examine the histories of all cases of Stokes-Adams disease that have been put on record. But it was soon found that this method of deciding the matter would be impracticable. For the number of cases that have been described is enormous,—very much larger than the recent collections would lead one to suppose.[38] And, furthermore, in the vast majority of instances the data given do not suffice to rule out heart-block, much less to prove that heart-block existed. This much may, however, be said: in not a single instance among the many cases that have been examined, has the description of a case of typical Stokes-Adams disease precluded the existence of heart-block. Indeed, it may be stated positively that in many instances the data sufficed for the diagnosis of heart-block.[39]

But if it is justifiable to choose from all of the cases that have been described only those that have been studied by methods of sufficient accuracy to permit of exact diagnosis, then it may be stated that all such cases of Stokes-Adams disease have proved to be instances of heart-block. It is true that in a few of these instances the exact condition was not recognized by the authors, indeed, one of the authors states that in his case the phenomena were not caused by heart-block. But in each case, had the proper interpretation of the tracings been made, the diagnosis of heart-block would have been reached.

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[38] Collective references to cases of slow pulse and cases of Stokes-Adams disease have been made by Prentiss, *Trans. of the Assn. of American Phys.*, 1889, iv, 120; Webster, loc. cit.; Edes, *Trans. of the Assn. of American Phys.*, 1901, xvi, 521; Jaquet, *Deutsches Arch. für klin. Med.*, 1902, lxxii, 77. Osler (*Lancet*, 1903, ii, 516) has described twelve previously unreported cases of Stokes-Adams disease.

[39] The criteria employed in reaching this decision are laid down in the section on diagnosis. As an example of the difficulties in the way of using this method of analyzing cases, the following may be mentioned. A case of Stokes-Adams disease was described by Figuet (Thesis, Lyon, 1882) as a typical instance of "rythmé couplé." About one year later, Chauveau (the full report of this case appeared in *Rev. de méd.*, 1885, v, 161) demonstrated that the case was one of heart-block. This, by the way, is the first demonstrated instance of heart-block in man.

The cases which have been used as a basis for the statements of the foregoing paragraph are collected in the accompanying table.

TABLE XVII.

CASES OF STOKES-ADAMS DISEASE WHICH ARE ACCOMPANIED BY DATA SUFFICIENT FOR AN EXACT DIAGNOSIS OF THE CARDIAC CONDITION. (INCLUDING TWO CASES OF HEART-BLOCK WITHOUT SYNCOPAL ATTACKS.)

| Author. | Where Described. | Diagnosis of Author. | Etiology. | Remarks. |
|---|---|---|---|---|
| 1. Chauveau. | *Rev. d. méd.*, 1885, v, 161. | Heart-block. | — | Tracings. |
| 2. Vaquez and Bureau. | *C. r. Soc. d. Biol.*, 1893, v, 169. | " | Abdominal traumatism. | Tracings. No syncopal attacks. |
| 3. Vaquez and Bureau. | *C. r. Soc. d. Biol.*, 1893, v, 169. | " | — | Tracings. |
| 4. His, Jr. | *Deutsches Arch. f. klin. Med.*, 1899, lxiv, 316. | " | — | Tracings. |
| 5. Hoffmann. | *Zeit. f. klin. Med.*, 1900, xli, 357. | " | Anæmia (?) | Tracings and X-rays. |
| 6. Webster. | *Glasgow Hosp. Rep.*, 1900, iii, 413. | No diag. | — | Tracings of radial pulse only, but these show two perfect and independent rhythms. |
| 7. Wenckebach. (2 cases ?) | *Die Arhythmie*, 1903. | Heart-block. | Arterio-sclerosis (?) | Tracings. |
| 8. Jaquet. | *Deutsches Arch. f. klin. Med.*, 1902, lxxii, 77. | Extra-systoles. | Syphilis. | Tracings misinterpreted: two perfect and independent rhythms in cardiogram. |
| 9. Lichtheim. | *Deutsche med. Woch.*, 1902, xxviii, 69. | Heart-block. | Arterio-sclerosis (?) | Tracings—3 : 1 rhythm. |
| 10. Mackenzie. | *Brit. Med. Jour.*, 1902, ii, 1411. | " | Influenza. | Tracings. No syncopal attacks: but condition acute, and patient constantly in bed. |
| 11. Gerhardt. | *Schmiedeberg's Arch.*, 1904, li, 11. | " | — | Tracings. |
| 12. Schmidt. | Quoted by Finckelnburg. | " | (?) | X-rays. |
| 13. Finckelnburg | *Deutsches Arch. f. klin. Med.*, 1905, lxxxii, 586. | " | — | Tracings. |

## THE DIAGNOSIS OF HEART-BLOCK—STOKES-ADAMS DISEASE.

Undoubtedly every stage of interference to the passage from auricles to ventricles of the impulse which normally causes the heart to beat should be included under the head of heart-block. However, that stage in which the block just suffices to increase the length of the intersystolic period would not give rise to symptoms, and consequently it would be but rarely found, merely because it would be but rarely looked for. But any stage beyond this would be characterized by the existence of perfectly regular auricular beats, some of which would not be followed by ventricular beats. In the early stages there would be occasional *ventricular silences.* Later these might recur regularly. Then every other auricular impulse might fail to stimulate the ventricles, and so on until none of the auricular impulses would arrive in the ventricles. Then the auricles and ventricles would beat totally independently of one another, the ventricles much more slowly than the auricles, but each with perfectly regular rhythms, barring, of course, the existence of those extraneous circumstances which in other states give rise to cardiac arhythmia.

In order to make the diagnosis of heart-block it is, therefore, necessary to show that the ventricles more or less regularly fail to respond to one or more of the regularly recurring auricular impulses.

Naturally, the most certain method of arriving at a correct diagnosis consists in making tracings of one or of all of the following: apex beat, jugular pulse, and arterial pulse. When the case is perfectly clear cut, and the signs distinct, it is conceivable that any one of the above tracings might suffice for the diagnosis. For the marks of both auricular and ventricular activities may, as in the case of our patient, be evident on all of them. If there can be distinguished in any one of these tracings the marks of two perfectly regular rhythms the diagnosis of heart-block may be made with reasonable certainty.

We can at present recall but two possible, though rare, sources of error. (1) In the form of arhythmia to which the name alternating pulse has been given, two perfectly regular rhythms are stated to occur: a strong heart-beat is regularly followed by a weak heart-beat, and these may be equally spaced. But in this condition the signs which are dependent upon the feebler beats are much too distinct to be confused with the signs produced by the beats of the auricles alone. (2) Extra-systoles of ventricular origin might rarely simulate heart-block. We have recently had the opportunity of studying a case of this kind. The tracings obtained are shown in Fig. 27. At the time this tracing was made a ventricular extra-systole occurred after every fourth normal systole. The signs of the extra-systoles are perfectly clear in the cardiogram. The cardiogram of a case of this kind could, therefore, never give rise to ambiguity. But at first sight the main waves on the jugular pulse appear to be spaced by equal distances. However, measurement shows that this is not the case; the larger waves occur somewhat earlier than the smaller waves. The larger waves are produced by auricular contractions, the stimuli for which are derived from the retrograde impulses generated by the ventricular extra-systoles. It is, of course, possible that the retrograde impulses might arrive in the auricles at such a time as not to interfere with the normal auricular rhythm. In such a case the auricular rhythm would be perfect, while in the peripheral arteries there might be no wave to correspond with one of the auricular beats. Therefore, in a case of this kind, if tracings of the venous and arterial pulses alone were made, there might be some difficulty in arriving at a diagnosis; but this difficulty would disappear upon comparing the arterial and venous tracings with the cardiogram. Therefore ventricular extra-systoles need not give rise to confusion in the diagnosis of heart-block.

But in most instances it should be possible to arrive at a diagnosis of heart-block with the aid of the usual clinical methods alone. Thus, after the author had become thoroughly familiar with the condition, there was usually but little difficulty in determining the auricular and ventricular rates by inspection of the veins of the neck. And we believe that in most cases when the block is complete or partial, with at least a 2 : 1 rhythm, it will be found possible to distinguish the two regular rhythms in the veins of the neck—rhythms independent of one another in complete block or with a definite ratio in partial block. Upon inspection of the apex-beat the same double rhythm might be made out in favorable cases, but the movements produced by the beats of the auricles would, at best, be very indistinct. Upon auscultation at the apex the same double rhythm might be discovered: the sounds associated with the movements of the heart synchronous with the pulse-waves in the peripheral arteries would be loud and distinct; between these might be heard the very feeble and distant sounds produced by the beats of the auricles.

So far as we know, the only condition that might be confused with this is one to which we have already referred, namely, extra-systoles of ventricular origin. But Hochhaus and Quincke[40] have shown, and we have recently had the opportunity of confirming this in a single case, that the apex-beat and heart sounds associated with an extra-systole are fully as distinct as those of a normal systole.[41]

The results which we have obtained in our animal experiments and during the recovery of our case of Stokes-Adams disease seem to indicate that an increase in the length of the intersystolic period, together with an occasional ventricular silence, will probably be found to be the first sign of beginning Stokes-Adams disease. These disturbances could be determined only by means of carefully made tracings. The methods of interpreting such tracings have been so fully considered by Wenckebach[42] and by Mackenzie[43] that nothing can be added to their descriptions.

Finally, we believe that the Stokes-Adams syndrome of slow pulse with syncopal attacks, during which the pulse is unusually slow, will in itself be found to be pathognomonic of heart-block. At least no other pathological entity can be conceived which could develop just this combination of symptoms. But cases of heart-block without syncopal attacks have been described, and the question therefore arises—Should a distinction be made between heart-block without syncopal attacks and heart-block with syncopal attacks? It is, of course, possible that the lesion which causes the heart-block might be different in the two groups. But clinical records indicate that they merely represent two stages of the same disease. Cases have been described in which the syncopal attacks made their first appearance long after the bradycardia. In our second case it required about one year for the full development of the Stokes-Adams syndrome. And it is well known that the frequency of the syncopal attacks increases as the end approaches. We therefore believe it will be shown that the distinction between heart-block with and without syncopal attacks is an arbitrary one.

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[40] Hochhaus and Quincke, *Deutsches Arch. für klin. Med.*, 1894, liii, 415.

[41] Many cases of slow pulse have been described as instances of so-called hemisystole. But we maintain with Hering (*Deutsche med. Woch.*, 1903, xxxii, 281) that hemisystole is not a well-characterized physiological condition and that there is no demonstrated case of hemisystole on record. The first supposed instance described, that of Leyden (*Virchow's Archiv*, 1868, xliv, 365), is clearly one of ventricular extra-systole.

[42] Wenckebach, *Die Arhythmie*, etc., 1903.

[43] Mackenzie, *British Med. Jour.*, 1905.

## THE ÆTIOLOGY OF STOKES-ADAMS DISEASE.

Although many cases of Stokes-Adams disease have come to autopsy, and although many lesions have been discovered in such cases, these lesions have been very variable and in practically no case has it been possible to show any direct relation between the lesion and the symptoms presumably caused by it. Among other things, there have been described sclerosis of the cerebral arteries and of the coronary arteries, fatty degeneration of the heart, tumors pressing upon the vagi, fracture of the odontoid process, with enlargement of the middle cervical ganglion, etc. In some cases nothing has been found. From the evidence which has been presented in this paper, the conclusion is warranted that a lesion which causes heart-block must be located in or near the auriculo-ventricular bundle: in it, if it is a degenerative lesion; near it, if it acts by compressing the bundle. We have been able to find but one case of Stokes-Adams disease in which there existed a lesion that might have caused heart-block by compressing the auriculo-ventricular bundle. Sendler [44] describes a case with a pulse of 40 and typical syncopal attacks, in which there was found at autopsy a cartilaginous mural tumor the size of a walnut. It was situated about one centimeter below the ostium of the pulmonary artery, but did not touch the valves. No other lesion has been found here, probably because it has never been carefully looked for. As a discussion of this matter must therefore be hypothetical and unsatisfactory, we shall refer to but two of the possibilities, one of which is of more than passing interest.

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[44] Sendler, *Cent. für klin. Med.*, 1892, p. 642. Two cases of Stokes-Adams disease have recently been described in which it is probable that a lesion in the auriculo-ventricular bundle was the cause of the trouble: Stengel, *Amer. Jour. of the Med. Sciences*, 1905, cxxx. 1083, and Hay, *Brit. Med. Jour.*, 1905, ii, 1034.

It may have been a coincidence that both of the cases that have come under our observation have given clear histories of syphilis. It may also have been a coincidence that one of the cases which has been under observation for a sufficiently long interval of time has practically recovered under treatment with potassium iodide, recommended by Dr. Osler. But when it is recalled that Jaquet's patient, who also gave a syphilitic history, was distinctly benefited while under an antisyphilitic treatment,[45] it seems to be more than likely that syphilis may be one of the ætiological factors of Stokes-Adams disease. But if syphilis causes heart-block, it certainly is not the only cause; at least it does not seem to be mentioned more frequently in the histories of cases of Stokes-Adams disease than in the histories of other diseases.

The second possibility is one for which no basis has been obtained by observation. The close relation which the mesial leaflet of the tricuspid valve bears to the auriculo-ventricular bundle of His makes it appear as probable that disease of this leaflet might, by putting His's bundle under tension, interpose a block to the passage of normal impulses from auricles to ventricles. At any rate, the condition of this leaflet should be carefully determined in all cases of Stokes-Adams disease that come to autopsy.

## THE TREATMENT OF STOKES-ADAMS DISEASE.

It is not the purpose of the author in writing this section to detail the symptomatic treatment of Stokes-Adams disease. It is intended to refer here to but two phases of the subject which have been encountered during the course of this investigation.

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[45] Jaquet, loc. cit.

In the first place, the apparent recovery of one of the cases under treatment with potassium iodide should here be recalled. For this result makes it desirable to emphasize the importance of determining the presence of, and of treating, syphilis in cases of Stokes-Adams disease. But the fact that Hoffmann saw a case recover under repeated administrations of oxygen makes it additionally desirable to determine in these cases of recovery the parts played by treatment and the natural defences of the individual.

The second point to which reference should be made is the effect which the administration of atropine had in temporarily stopping the attacks of syncope. Although the results of this research justify the statement that atropine will not increase materially the slow pulse-rate of heart-block, still it is possible that in mild cases atropine, by steadying the pulse-rate, might tend to ward off attacks of syncope.

## CONCLUSIONS.

The facts collected in the third part of this paper justify the following conclusions:

(1) All of the cardinal symptoms of Stokes-Adams disease may be duplicated by heart-block resulting from a lesion in or near the auriculo-ventricular bundle of His, and by this alone.

(2) No typical case of Stokes-Adams disease has been described in which heart-block might not have been the cause of the trouble.

(3) It can be shown that all cases of Stokes-Adams disease which have been studied by sufficiently accurate methods were cases of heart-block.

(4) It would appear that heart-block without and with syncopal attacks are two stages of the same disease process.

# APPENDIX.

## PROTOCOLS OF EXPERIMENTS OF PART II.

### *Series A.—Experiments in which Ligatures Were Used.*

Exp. 1. Feb. 1, 1905. As soon as ligature was passed and before it was tied the whole heart went into fibrillary contractions from which it did not recover.

*Autopsy.*—Ligature entered heart anterior to membranous septum, passed directly into the right ventricle, and out of it posterior to the membranous septum.

Exp. 2. Feb. 7, 1905. Ligature passed and tied without any effect on the heart.

*Autopsy.*—Ligature entered right ventricle anterior to membranous septum, passed upward into the right auricle and emerged from it about 1 cm. from the root of the aorta.

Exp. 3. Feb. 9, 1905. Before passing ligature obtained partial block with 2: 1 rhythm upon stimulating vagus with coil at fifteen; with the coil at thirteen complete block was obtained. The first ligature was passed without effect on the heart. The second ligature was entered at about the same place as the first, but was brought out somewhat farther posteriorly. As soon as it was passed and before it was tied the auricles went into fibrillary contractions. The ventricles did not stop; but continued beating with a very slow rate. During this period, which lasted about ten minutes, the ventricles could not be inhibited even with the coil at zero. Then the auricles began to beat, but with a rate very much faster than that of the ventricles and the rhythms were independent of one another, auricle beating about three times to ventricle once. At this time stimulation of the vagus with the coil at thirteen gave complete inhibition of the auricles, but had absolutely no effect upon the ventricles. Strongest stimulation of the vagus (coil at zero) had no influence upon the ventricles.

This condition lasted for about a half-hour when the complete block changed into a partial block with a 3 : 1 rhythm. Now stimulation of the vagus with the coil at fifteen gave complete inhibition of the whole heart which could be maintained for some time. The partial block gradually disappeared through a 2 : 1 rhythm. When the normal rhythm had returned it was not possible to produce a partial block by stimulation of the vagus with any strength of stimulus. Now the second ligature, which had simply been passed, was tied, but without influence on the condition of the heart.

*Autopsy.* On opening the heart both ligatures slipped out, therefore the positions they had occupied could not be determined.

Exp. 4–7. All failed. In two the heart was torn while making the preliminary dissection, and hæmorrhage prevented further work. In one the only fine needle possessed broke. In another the ligature was passed and tied with negative results. Autopsy showed that the ligature had not passed into ventricular muscle at all; it had passed through auricular tissue only.

## *Series B.—Experiments with Vise Clamp.*

Exp. 8. Feb. 15, 1905. Clamp was put in place without difficulty and without disturbing normal sequence of heart-beat. Tracings of auricular and ventricular movements were made with the myocardiograph of Roy and Adami; there was no time record, hence the results can be expressed only relatively.

(A) Stimulation of vagus: complete inhibition of whole heart.

(B) Clamp tightened until complete block came on. The marks of the ventricular contractions can be seen on auricular tracing. From this it may be seen that the ventricular systole falls in any phase of the auricular cycle. In the course of 47 auricular contractions the ventricles contract 7 times.

(C) Now stimulation of vagus gave complete inhibition of the auricles, but the ventricles continued to beat. Before stimulation of vagus one ventricular systole occurred regularly in 7 mm. of tracing. During vagus stimulation one ventricular systole occurred in 9 mm.; after inhibition one ventricular systole in 8 mm.

(D) Second stimulation of vagus. Complete inhibition of auricles. Ventricular rate increased: one ventricular systole in 3 mm. First ventricular cycle after inhibition 10 mm., then normal rate: one ventricular systole in 7 mm.

(E) Third stimulation of vagus. Complete inhibition of the auricles, ventricular rate increased more or less gradually, becoming one ventricular systole in 2.5 mm. After inhibition the distances between successive ventricular systoles = 10, 8, 7.5, 7, 7, 7.5, 7.7 mm.

(F) Unclamped: normal sequence returned.

The whole experiment was repeated with similar results.

*Autopsy.*—Posterior arm of clamp entered the left ventricle posterior to the membranous septum and through the auriculo-ventricular junction. It passed anteriorly, piercing one of the leaflets of the aortic valve. The anterior arm entered the right ventricle anterior to the membranous septum, about 3 mm. below the auriculo-ventricular junction and ran posteriorly in the cavity of the right ventricle.

Exp. 9. Feb. 17, 1905. While passing the clamp the auricles twice went into fibrillary contractions, but recovered. To make room for the myocardiograph the clamp was drawn to the left side under some tension. Without warning, the ventricles went into fibrillation, and the dog died.

*Autopsy:* Both arms of the clamp entered the heart at the auriculo-ventricular junction, the posterior one as in preceding experiment; the anterior one pierced the middle of the membranous septum.

Exp. 10. Feb. 21, 1905. Auricular contractions recorded by tambours, ventricular contractions and blood-pressure by mercury manometer connected with carotid artery. Time in seconds inscribed on base-line. Passed clamp with no effect. Clamped with no effect. However, stimulation of vagus was now associated with a remarkable phenomenon. The heart could be inhibited as easily as before clamping, but while the heart was recovering from inhibition, both the auricles and ventricles went into contractions which resembled fibrillation very closely. This was repeated several times.

*Autopsy.*—The anterior arm of clamp passed through the muscular septum anterior to the membranous septum. The posterior arm entered the aorta too high and at the posterior edge of the membranous septum.

TABLE XVIII.

| Procedures and Remarks. | | No. of Vs. | Duration in Sec. | V. Rate per Min. | No. of As. | Duration in Sec. | A. Rate per Min. | V | Max. Pres. | Min. Pres. |
|---|---|---|---|---|---|---|---|---|---|---|
| Stim. of vagus, medium. | Before. | 8 | 13.8 | 34.8 | 32 | 14.8 | 130 | 3.7 | 41 | 21.5 |
| | During. | 6 | 12+ | 30 | 0 | — | — | — | 34 | 17+ |
| | After. | 7 | 12.2 | 34.4 | 24 | 11.6 | 124 | 3.6 | 46 | 28 |
| Same, stronger. | Before. | 5 | 8.1 | 37 | 16 | 7.3 | 131 | 3.5 | 48 | 32.5 |
| | During. | 5 | 7.7 | 38.9 | 0 | — | — | — | 42 | 32.5 |
| | After. | 4 | 7 | 34.2 | 12 | 6.2 | 116 | 3.4 | 42.5 | 27 |
| Same, weak. (As. cannot be counted). | Before. | 5 | 8.4 | 35.7 | 17 | 8 | 127.5 | 3.6 | 42 | 28.5 |
| | During. | 6 | 10.2 | 35.3 | 0 | — | — | — | 36 | 25 |
| | After. | 3 | 5.1 | 35.3 | — | — | — | — | 40 | 27.5 |
| Same, very strong. (As. cannot be counted.) | Before. | 5 | 8.6 | 34.9 | 22 | 10.6 | 124.5 | 3.6 | 42 | 32 |
| | During. | 8 | 14.6 | 32.9 | 0 | — | — | — | irreg. | |
| | After. | 4 | 7.3 | 32.9 | — | — | — | — | " | |
| Compression of abd. aorta. | Before. | 5 | 9.2 | 32.6 | 18— | 9.2 | 117 | 3.6 | 43 | 34 |
| | During. | 7 | 13.1 | 32.1 | 25+ | 13.1 | 115 | 3.6 | 53 | 41 |
| | After | 4 | 7.6 | 13.6 | 14.5 | 7.6 | 114.5 | 3.6 | 38.5 | 30 |
| Same. | Before. | 6 | 12.1 | 29.8 | 22 | 12.1 | 109 | 3.7 | 48 | 38 |
| | During. | 8 | 16.1 | 29.8 | 29.7 | 16.1 | 111 | 3.7 | 62 | 49 |
| | After. | 3 | 6 | 30 | 11 | 6 | 110 | 3.7 | 44 | 36 |
| Compression of thor. aorta. | Before. | 5 | 10.5 | 28.6 | 19.5 | 10.5 | 111.5 | 3.9 | 42.5 | 33 |
| | During. | 6 | 12.9 | 27.9 | 24 | 12.9 | 112 | 4 | 65 | 47 |
| | After. | 5 | 11.2 | 26.8 | 21 | 11.2 | 112.5 | 4.2 | 39 | 31 |
| | | 7 | 15.2 | 27.6 | 28 | 15.2 | 110.5 | 4 | 42 | 33 |
| Stimulation of vagus, weak. | Before. | 4 | 8.6 | 27.9 | 16 | 8.6 | 111.5 | 4 | 41 | 32 |
| | During. | 6 | 13.1 | 27.5 | greatly reduced | — | — | — | 38 | 32 |
| | After. | 4 | 8.8 | 27.3 | 15.5 | 8.8 | 106 | 3.9 | 39.5 | 31.5 |
| Same, medium. | Before. | 6 | 13.1 | 27.5 | 22 | 11.9 | 111 | 4 | 40.5 | 32 |
| | During. | 6 | 13.7 | 26.3 | 0 | — | — | — | 37 | 32 |
| | After. | 3 | 6.8 | 26.5 | 15 | 8.9 | 101 | 3.8 | 39 | 30.5 |
| Same, strong. | Before. | 5 | 11.2 | 26.8 | 17 | 9.4 | 108 | 4 | 38 | 30 |
| | During. | 5 | 11.5 | 26.1 | — | — | — | — | irreg. | |
| (A. does not record.) No procedure. | | 6 | 21.1 | 17.1 | — | — | — | — | 22 | 16 |

Exp. 11. Feb. 23, 1905. While passing the second half of clamp the first half slipped out. The first half of the clamp was reinserted blindly through the hæmorrhage. When the clamp was tightened no block resulted.

*Autopsy.*—Both arms are above the auriculo-ventricular junction and embrace auricular tissue only.

Exp. 12. Feb. 24, 1905. I had some difficulty in adjusting the clamp; one arm slipped out of the heart. The hæmorrhage that resulted made it difficult to reinsert clamp. After readjustment, tightening of the clamp had no influence on sequence.

*Autopsy.*—The clamp embraced auricular muscle and the wall of the aorta only.

Exp. 13. Feb. 24, 1905. While introducing the clamp I got momentary heart-block, but tightening the clamp had no influence upon sequence nor upon the ease with which the whole heart could be inhibited.

*Autopsy.*—Both arms entered the heart above the line marking the auriculo-ventricular junction and embraced auricular tissue and aortic wall only.

Exp. 14. Feb. 25, 1905. Posterior arm of clamp inserted without mishap. At the instant the anterior arm was passed, and before tightening, complete heart-block came on and persisted throughout the experiment, three quarters of an hour. Auricular contractions were recorded with tambours; ventricular contractions and blood-pressure with mercury manometer in the carotid artery; time is marked in seconds on base-line.

*Autopsy.*—Posterior limb of clamp was in left ventricle some distance posterior to membranous septum. The anterior limb entered the left ventricle just below the aortic valves, perforated the middle of the membranous septum, and entered the right ventricle; turned posteriorly and entered the muscular septum about 2–3 mm. posterior to the posterior edge of the membranous septum, and the point lay in the left ventricle. Hence the portion of the muscular septum between the posterior edge of the membranous septum and a point 2–3 mm. posterior to this was gathered on the anterior limb of the clamp. Both coronary arteries were patent.

The analysis of the tracings is given in Table XVIII.

## *Series C.—Experiments with Fishhook Clamp.*

Exp. 15. Feb. 28, 1905. Large dog. Clamp was passed and experiment begun at 4.30 P.M. and concluded at 6.00 P.M. Details of the experiment are given in Table XIX. At one time the clamp was left tightened for over 45 minutes, and upon loosening it, normal sequence returned. At the end of the experiment, while killing the animal by asphyxia and at a time when the animal was all but dead, the clamp was loosened and normal sequence returned and persisted until the heart stopped.

*Autopsy.*—Hook entered the aorta exactly at its root, at the upper and posterior edge of the membranous septum. It pierced the muscular septum about 3 mm. below, and posterior to, the posterior edge of the membranous septum.

Exp. 16. March 2, 1905. Small dog. Clamp adjusted on heart, but clamping produced no block.

*Autopsy.*—Clamp was placed about as in preceding experiment. Negative result was probably due to fault in clamp. A large space close in toward the hook could not be obliterated; important tissue might have been in that place. A new clamp was made in which there could be no dead space. This clamp was used in all subsequent experiments.

Exp. 17. March 3, 1905. Very large dog. While dissecting alongside of the aorta there was considerable hæmorrhage which was finally controlled. Clamp was introduced without producing block. Upon tightening the clamp complete block came on promptly, auricular to ventricular rate being about 4 : 1. While preparing to insert coronary sound the dog died.

## TABLE XIX.

| Procedures and Remarks. | | No. of Vs. | Duration in ⅕ Sec. | V. Rate per Min. | No. of As. | Duration in ⅕ Sec | A. Rate per Min. | $\frac{A}{V}$ | Max. Pres. | Min. Pres. |
|---|---|---|---|---|---|---|---|---|---|---|
| Clamp open. | | | | | | | | | | |
| Stim. of vagus, strong. | Before. | 45 | 59.5 | 227 | 45 | 59.5 | 227 | 1 | 75 | 73 |
| | During. | 3 | 25.6 | 35.2 | 0 | — | — | — | 52 | 35 |
| | After. | 50 | 61.2 | 245 | 50 | 61.2 | 245 | 1 | 71 | 69 |
| After clamping. | | | | | | | | | | |
| Stim. of vagus, same. | Before. | 20 | 86 | 69.8 | 62 | 86 | 216 | 3.1 | 59 | 49 |
| | During. | 13 | 58 | 67.2 | 0 | — | — | — | 48 | 38.5 |
| | After. | 20 | 78.6 | 76.3 | 61 | 78.6 | 233 | 3.05 | 55 | 47.5 |
| After unclamping. | | | | | | | | | | |
| Stim. of vagus, same. | Before. | 37 | 49.6 | 224 | 37 | 49.6 | 224 | 1 | 74 | 72 |
| | During. | 2 | 30.5 | 20 | 0 | — | — | — | — | 21 |
| | After. | 36 | 47 | 230 | 36 | 47 | 230 | 1 | 65 | 63 |
| Clamping and unclamping. | | | | | | | | | | |
| Clamping (stopped as soon as block appeared). | | 43 | 60.5 | 213 | 43 | 60.5 | 213 | 1 | 69 | 67 |
| The above passes immediately into: | | 14 | 39.2 | 107 | 28 | 39.2 | 214 | 2 | 63 | 60 |
| The above is followed by, 1-3:1, 2-2:1, then: | | 7 | 28.4 | 74 | 21 | 28.4 | 222 | 3 | 58 | 52 |
| Followed by 1-4:1(?), 2-3:1, then: | | 8 | 37.8 | 63.5 | 26.5 | 37.8 | 210 | 3.3 | 53 | 46 |
| Unclamped completely, | | 9 | 38 | 71 | 27 | 38 | 213 | 3 | 56 | 50 |
| Followed by 1-2:1, 1-3:1, 2-2:1, 1-3:1, then: | | 4 | 10.7 | 112 | 8 | 10.7 | 224 | 2 | 64 | 60 |
| Then: | | 20 | 27.8 | 216 | 20 | 27.8 | 216 | 1 | 70 | 68 |
| Clamped. | | | | | | | | | | |
| Effect of asphyxia (stoppage of art. resp.). | Before. | 10 | 43 | 69.8 | 30 | 43 | 209 | 3 | 59 | 53 |
| Approaching highest pressure. | | 8 | 31 | 77.4 | — | — | — | ? | 125 | 120 |
| Art. resp. resumed. | | | | | | | | | | |
| | Pressure falling. | 6 | 30.6 | 58 | 19 (?) | 30.6 | 201 | 3.4 (?) | | |
| | Pressure constant. | 12 | 58.8 | 61.2 | 46– | 58.8 | 235 | 3.8 | 86 | 78 |
| | Later. | 11 | 59 | 55.9 | 45 | 59 | 229 | 4.1 | 94 | 85 |
| Compression of abd. aorta. | Before. | 10 | 59 | 50.8 | 43 | 59 | 219 | 4.3 | 66.5 | 59 |
| | During. | 9 | 54 | 50 | 40 | 54 | 220 | 4.4 | 85 | 76 |
| Compression of abd. aorta. | Before. | 10 | 66 | 45.5 | 46.5 | 66 | 211 | 4.6 | 68 | 58 |
| | During. | 10 | 68.2 | 44 | 48 | 68.2 | 211 | 4.8 | 95 | 83 |
| | After. | 10 | 66 | 45.5 | 45 | 66 | 205 | 4.5 | 50 | 40 |
| Stim. of vagus: coil at o. | Before. | 10 | 67 | 44.3 | 45 | 67 | 201 | 4.5 | 58 | 49 |
| | During. | 5 | 47.5 | 31.6 | 0 | — | — | — | 46 | 35 |
| | After. | 10 | 59 | 51 | 45 | 59 | 229 | 4.5 | 56 | 48 |
| Same. | Before. | 10 | 68 | 44.1 | 47 | 68 | 207 | 4.7 | 61 | 51 |
| | During. | 5 | 48 | 31.2 | 0 | — | — | — | 47 | 36 |
| | After. | 10 | 59.6 | 50.3 | 45 | 59.6 | 226 | 4.5 | 56 | 47 |
| Effect of cutting both vagi. | Before. | 10 | 65.2 | 46 | 46 | 65.2 | 213 | 4.6 | 65 | 56 |
| | During (pres. at max.). | 10 | 62.4 | 48.1 | 51 | 62.4 | 245 | 5.1 | 119 | 108 |
| | Later. | 10 | 67.5 | 44.4 | 54 | 67.5 | 240 | 5.4 | 99 | 90 |
| After unclamping. | | 43 | 53.2 | 242 | 43 | 53.2 | 242 | 1 | 123 | 122 |
| After clamping. | | | | | | | | | | |
| Effect of asphyxia. | Before. | 10 | 56 | 53.6 | 45 | 56 | 241 | 4.5 | 87 | 80 |
| | During (pres. rising). | 10 | 59.2 | 50.7 | 48.5 | 59.2 | 246 | 4.8 | 147 | 137 |
| | During (pres. falling). | 8 | 55 | 43.6 | 44 | 55 | 240 | 5.5 | falling | |
| Art. resp. resumed (pres. almost at min.). | | 10 | 69 | 43.5 | 56 | 69 | 243 | 5.6 | 92 | 80 |
| (Pres. rising subsequently.) | | 10 | 62.2 | 48.2 | 50 | 62.2 | 241 | 5 | 116 | 107 |
| After cutting both splanchnics (long interval.) | | 5 | 77 | 19.5 | ? | | | | 25 | 17 |
| After unclamping. | | 30 | 62.6 | 114 | 30 (?) | 62.6 | 144 | 1 | 22 | |

*Autopsy*—Both coronaries patent. The hook had passed into the aorta, perforating the anterior edge of the posterior leaflet of the aortic valve; passed downward, entered the muscular septum just posterior to the membranous septum, and, running obliquely backwards and downwards, entered the right ventricle at a place behind the center of the mesial leaflet of the tricuspid valve.

The bit of tissue included in the grasp of the clamp was saved for histological examination.

Exp. 18. March 4, 1905. Small fox terrier. Preparation was the same as in preceding experiments. When clamp was put in place and tightened no block resulted. Hook was then withdrawn from the ventricular septum and reinserted in what was thought to be a better position. Now tightening gave complete block. The hook was unclamped immediately and I began to make tests, details of which are given in Table XX. The block did not disappear for some time; the first escape is given in the table.

*Autopsy.*—The hook was found to occupy the same position as in the preceding experiment. Both coronaries were patent. The tissue included in the clamp was excised for histological examination.

Exp. 19. March 6, 1905. Small dog.

(*a*) I tried to establish block by cooling; a brass tube of 2 mm. bore was bent so that it would fit snugly in the groove between the aorta and the right auricle. After putting it in place I circulated fluid at $-3°$ C. through it for 10 min. without effect upon the sequence of the heart-beat.

(*b*) Clamp was then adjusted on heart. While passing the hook there was a transitory 2 : 1 rhythm. Later (before preparations for making tracing had been completed) 2: 1 rhythm occurred. Now during very weak stimulation of the vagus the rhythm changed to 1: 1, returning to 2 : 1 upon cessation of stimulation. This was repeated several times. Complete block then gradually developed and persisted for about 3 hrs., although the clamp had not been tightened. After recovery from block, tightening of the clamp caused the block to return with great precision. This phenomenon, with other details, is recorded in Table XXI. At the close of the experiment while the block was complete the right coronary artery was plugged by Porter's method. This was associated with no perceptible change in the rate of the ventricles, although the artery was closed for more than 2 min. The heart continued to beat for some minutes after excision.

*Autopsy.*—The sound is in the right coronary artery. The clamp occupies practically the same position as in preceding experiment. The tissue included in the clamp was excised for histological examination.

Exp. 20. March 7, 1905. Small dog. While passing the hook complete block came on and persisted throughout the course of the experiment. At the close of the experiment the left coronary artery was plugged by Porter's method. Although no record was made of this test, it was perfectly obvious that the rate of the ventricular beat was not materially affected after 4 min. Then the artificial respiration was stopped with the coronary sound still in place. After 3 min. the heart was still beating, and it continued to beat for some time after the heart had been excised, even after opening the right and left ventricles. The details of the experiment are given in Table XXII.

TABLE XX.

| Procedures and Remarks. | | No. of Vs. | Duration in ⅕ Sec. | V. Rate per Min. | No. of As. | Duration in ⅕ Sec. | A. Rate per Min. | A/V | Max. Pres. | Min. Pres. |
|---|---|---|---|---|---|---|---|---|---|---|
| Tracing begun after escape from complete block. | | | | | | | | | | |
| Stimulation of accel. | Before. | 40 | 66 | 182 | 40 | 66 | 182 | 1 | 96 | 92 |
| | During. | 41 | 68 | 181 | 41 | 68 | 181 | 1 | 94 | 90 |
| Same, coil at 11. | Before. | 31 | 51.8 | 180 | 31 | 51.8 | 180 | 1 | 94 | 90 |
| | During. | 26 | 43.2 | 181 | 26 | 43.2 | 181 | 1 | 93 | 89 |
| Same, coil at 9. | Before. | 27 | 45.3 | 179 | 27 | 45.3 | 179 | 1 | 93 | 90 |
| | During. | 30 | 50.1 | 180 | 30 | 50.1 | 180 | 1 | 93 | 90 |
| Same, coil at 9. | Before. | 26 | 44 | 177 | 26 | 44 | 177 | 1 | 93 | 90 |
| | During. | 28 | 46.8 | 179 | 28 | 46.8 | 179 | 1 | 93 | 90 |
| Block developing spontaneously, probably from tension on clamp; first rhythm, 2 : 1, then alternately 1:1 and 2:1, and finally 2:1 | | | | | | | | | | |
| Stim. of accel., coil at 9. | Before. | 13 | 43.6 | 89.5 | — | — | — | 2 (?) | 90 | 80 |
| (Auricles not writing.) | During. | 13 | 44 | 89.7 | — | — | — | 2 (?) | 90 | 80 |
| Vacillation between 3:1 and complete block. | | | | | | | | | | |
| Stim. of vagus, coil at 13. | Before. | 8 | 43 | 55.8 | 25.3 | 43 | 177 | 3.2 | 79 | 58 |
| | During. | 8 | 42.8 | 56.1 | 20 | 42.8 | 140 | 2.5 | 75 | 56 |
| | After. | 7 | 37.8 | 55.6 | 21.8 | 37.8 | 173 | 3.1 | 78 | 58.5 |
| Stim. of vagus, coil at 9. | Before. | 9 | 48 | 56.2 | 28.5 | 48 | 178 | 3.2 | 79 | 58 |
| | During. | 9 | 49 | 54.9 | 0 | — | — | — | 64 | 51 |
| Stim. of accel., coil at 9. | Before. | 13 | 69 | 56.5 | 38 | 69 | 165 | 2.9 | 74 | 52 |
| | During. | 13 | 69 | 56.5 | 39 | 69 | 170 | 3 | 76 | 55 |
| | After. | 10 | 52.6 | 57 | 30.3 | 52.6 | 173 | 3 | 76 | 56 |
| Stim. of vagus, coil at 9. | Before. | 9 | 48 | 56.2 | 26.3 | 48 | 164 | 2.9 | 74 | 52 |
| | During. | 9 | 49 | 55.1 | 0 | — | — | — | 63 | 50 |
| | After. | 8 | 43 | 55.8 | 23 | 43 | 161 | 2.9 | 73 | 51 |
| Stim of accel., coil at 7. | Before. | 8 | 42 | 57.1 | 23.2 | 42 | 166 | 2.9 | 74 | 53 |
| | During. | 14 | 74.6 | 56.3 | 40.5 | 74.6 | 163 | 2.9 | 76 | 54 |
| | After. | 11 | 58 | 56.9 | 33.5 | 58 | 173 | 3 | 75 | 53 |
| Effect of asphyxia. | Before. | 10 | 55.8 | 53.8 | 29.7 | 55.8 | 160 | 2.97 | 69 | 51 |
| (Art. resp. stopped.) | 1st 10 Vs. | 10 | 55.8 | 53.8 | 29.3 | 55.8 | 157 | 2.93 | 69 | 51 |
| | Before rise of pres. | 10 | 56.8 | 52.8 | 29 | 56.8 | 153 | 2.9 | 67 | 50 |
| | Pres. rising. | ? | — | — | 30 | 61 | 148 | — | max.<br>107 | 97 |
| (Art. resp. resumed.) | Pres. falling. | ? | — | — | 31 | 58.4 | 159 | — | | |
| | Later. | | | | 31 | 58 | 160 | | | |
| After this V becomes irregular: groups of 2 to 3-2:1 alternating with 3:1, or complete block. Pulse and pressure too irregular for measurement. | | | | | | | | | | |
| Effect of asphyxia. | Before. | 10 | 57.6 | 52.1 | 33.3 | 57.6 | 173 | 3.3 | 77 | 66 |
| | Art. resp. stopped. | 10 | 58 | 51.7 | 33.5 | 58 | 173 | 3.35 | 80 | 70 |
| | Pres. rising. | 10 | 57.6 | 52.1 | 32.5 | 57.6 | 169 | 3.25 | 84 | 74 |
| | Pres. constant. | 10 | 57.8 | 51.9 | 31 | 57.8 | 161 | 3.1 | 83 | 73 |
| Art. resp. resumed. | Pres. falling. | 10 | 58.2 | 51 | 31.7 | 58.2 | 162 | 3.2 | 77 | 68 |
| Stim. of vagus, coil at 9. | Before. | 12 | 42 | 85.7 | 24 | 42 | 171.4 | 2 | 89 | 84 |
| (Occasional As'.) | During. | 4 | 34.2 | 35.1 | — | — | — | — | — | 41 |
| | After recovery of auricles.* | 29 | 56.2 | 155 | 29 | 56.2 | 155 | 1 | 91.5 | 90 |
| Stim. of accel., coil at 9. | Before. | 25 | 44 | 170 | 25 | 44 | 170 | 1 | 73.5 | 72 |
| | During. | 26 | 50 | 156 | 26 | 50 | 156 | 1 | 76.5 | 75 |

* At cessation of stimulation the auricle gave two feeble beats followed by a very strong one, after which it went into a condition resembling fibrillary contractions for about 100/5 sec. When it resumed its normal condition its contractions were at first powerful, but they rather rapidly diminished in height (for about 25/5) and then slowly increased to normal size. At cessation of stimulation the ventricles began to beat with a rate that was faster than before stimulation, and within a few beats the pressure was at its maximum (98–96). When the auricles went into fibrillary contractions the ventricular rhythm became very irregular. It was slowest just before the auricles recovered as with inhibition. When the auricles began to beat the ventricles followed them with a 1: 1 rhythm.

*Autopsy.*—The sound is in the left coronary. Position of the clamp is about as in preceding experiments.

Exp. 21. March 8, 1905. Large dog. Insertion of the hook was not associated with any disturbance in sequence. On clamping to determine if the clamp was in the right place, complete block came on at once. At first the ventricular rate was very slow indeed, but it gradually increased until the ventricular rate usual for heart-block obtained. This phenomenon was observed a second time during the course of the experiment. For details, see Table XXIII.

TABLE XXI.

| Procedures and Remarks. | | No. of Vs. | Duration in ⅕ Sec. | V. Rate per Min. | No. of As. | Duration in ⅕ Sec. | A. Rate per Min. | A/V | Max. Pres. | Min. Pres. |
|---|---|---|---|---|---|---|---|---|---|---|
| After adjusting clamp complete block developed. | | | | | | | | | | |
| Stim. vagus, weak. | Before. | 15 | 65.7 | 68.5 | 35.5 | 65.7 | 157.5 | 2.3 | 81 | 76 |
| | During. | 18 | 79 | 68.3 | 42 | 79 | 159.4 | 2.3 | 74 | 68 |
| Stim. vagus, strong. | Before. | 16 | 70.6 | 68– | 37.7 | 70.6 | 160.2 | 2.3 | 76 | 70 |
| | During. | 11 | 52 | 63.5 | few. | — | — | — | 63 | 57 |
| Left vagus cut, rt. vagus cooled to −3° C. | | 17 | 74.3 | 68.6 | 39.8 | 74.3 | 160.7 | 2.3 | 81 | 75 |
| Stim. rt. vagus central of cooled area. | | | | | | | | | | |
| | Before. | 10 | 44.5 | 67.4 | 24 | 44.5 | 161.8 | 2.4 | 84 | 79 |
| | During. | 12 | 55.4 | 65 | few | — | — | — | 66 | 61 |
| | After. | 9 | 40.7 | 66.3 | 21.3 | 40.7 | 157 | 2.4 | 77 | 71 |
| Same. | Before. | 10 | 44.8 | 67 | 24.6 | 44.8 | 164.7 | 2.5 | 82 | 75 |
| | During. | 13 | 59.4 | 65.6 | 27 | 59.4 | 136.4 | 2.1 | 66 | 60 |
| Same. | Before. | 11 | 50.8 | 64.9 | 26.8 | 50.8 | 158.3 | 2.4 | 81 | 74 |
| | During. | 13 | 61 | 63.9 | 31 | 61 | 152.5 | 2.4 | 68 | 62 |
| Stim. rt. vagus after warming. | Before. | 10 | 46.8 | 64.1 | 24.6 | 46.8 | 157.7 | 2.5 | 87 | 80 |
| | During. | 13 | 63.4 | 61.5 | few | — | — | — | 72 | 64 |
| Stim. accel. several times—negative. | | | | | | | | | | |
| Cut rt. vagus. | Before. | 15 | 68.8 | 65.4 | 35.3 | 68.8 | 153.9 | 2.35 | 87 | 79 |
| | After. | 16 | 74 | 64.8 | 37.7 | 74 | 152.8 | 2.4 | 85 | 76 |
| Block passed off spontaneously: now stim. of vagus gave complete inhib. of A and V. | | | | | | | | | | |
| Before clamping. | | 17 | 55.2 | 92.4 | 17 | 55.2 | 92.4 | 1 | 54 | 51 |
| Clamping (occasional 2: 1, then:) | | 12 | 68.3 | 52.7 | 24 | 68.3 | 105.4 | 2 | 50 | 43 |
| Now stim. of vagus gives complete inhib. of A and V.* | | | | | | | | | | |
| Clamping. | | 9 | 66.2 | 40.8 | 20.4 | 66.2 | 92.5 | 2.3 | 47 | 36 |
| Stim. of vagus. | | 9 | 69.2 | 39 | 0 | — | — | — | 37 | 30 |

*Autopsy.*—Weight of heart, 94 grm. The hook entered the aorta a little to the right of the line of junction of the right and posterior leaflets of the aortic valve and 8 mm. below the uppermost point of the line of attachment of the leaflets. It entered the muscular septum 2 mm. posterior to the above-mentioned line of junction and on the line of attachment of the lower edge of the posterior leaflet with the muscular septum. It passed obliquely through the septum and appeared in the right ventricle just behind the middle point of e mesial leaflet of the tricuspid valve, about 3 mm. below the auriculo-ven-icular junction. The tissue included in the clamp was saved for histological xamination.

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* Fibrillary contractions of auricles occurred for a short time immediately after recovery from inhibition.

TABLE XXII.

| Procedures and Remarks. | | No. of Vs. | Duration in ⅕ Sec. | V. Rate per Min. | No. of As. | Duration in ⅕ Sec. | A. Rate per Min. | V\|A | Max. Pres. | Min. Pres. |
|---|---|---|---|---|---|---|---|---|---|---|
| timulation of vagus. | Before. | 8 | 50.2 | 47.8 | 26.4 | 50.2 | 157.7 | 3.3 | 94 | 88 |
| | During. | 6 | 39.8 | 45.2 | 0 | — | — | — | 77 | 72 |
| Stim. of accel. | Before. | 15 | 86.6 | 51.96 | 47.8 | 86.6 | 165.6 | 3.2 | 81 | 72 |
| | During max. accel. | 11 | 61 | 54.59 | 36.5 | 61 | 179.5 | 3.3− | 79 | 70 |
| | After. | 7 | 38.4 | 54.7 | 22.7 | 38.4 | 177.4 | 3.24 | 82 | 73 |
| Stim. of accel. | Before. | 11 | 59.3 | 55.65 | 33.3 | 59.3 | 168.5 | 3.03 | 74 | 66 |
| | During max. accel. | 13 | 65.4 | 59.6 | 41.3 | 65.4 | 189.4 | 3.18 | 82 | 74 |
| | After. | 7 | 35.2 | 59.66 | 21.6 | 35.2 | 184.1 | 3.09 | 71 | 64 |
| Stim. of accel. | Before. | 10 | 51.8 | 57.9 | 28.9 | 51.8 | 166.8 | 2.88 | 73 | 67 |
| | During max. accel. | 12 | 57.5 | 62.6 | 36.9 | 57.5 | 192.5 | 3.07 | 77 | 71 |
| | After. | 12 | 56.8 | 63.4 | 34.8 | 56.8 | 183.8 | 2.9 | 68 | 63 |
| Ligation of both vagi. | Before. | 11 | 53.7 | 61.4 | 29 | 53.7 | 162 | 2.64 | 68 | 62 |
| | After. | 11 | 53.4 | 61.8 | 29.3 | 53.4 | 164.6 | 2.66 | 60 | 55 |
| Stim. of accel. | Before. | 11 | 53.3 | 61.9 | 31.5 | 53.3 | 177.3 | 2.86 | 57 | 52 |
| | During. | 10 | 48.3 | 62.1 | 23.5 | 48.3 | 146 | 2.35 | 64 | 58 |
| | | 10 | 47.8 | 62.7 | 29.3 | 47.8 | 183.9 | 2.93 | 66 | 60 |
| | After. | 14 | 65.8 | 63.8 | 39.8 | 65.8 | 181.4 | 2.84 | 54 | 49 |
| At beginning of stim. auricles were slightly inhibited (escape) but V. slightly accelerated and pulse pressure and blood pressure increased. | | | | | | | | | | |
| Stim. of accel. | Before. | 10 | 49.2 | 61 | 26.2 | 49.2 | 159.7 | 2.62 | 57 | 51 |
| | During. | 17 | 82.6 | 61.7 | 45 | 82.6 | 163.4 | 2.65 | 61 | 56 |
| | After. | 16 | 79 | 60.8 | 42.6 | 79 | 161.8 | 2.66 | 54 | 49 |

Exp. 22. March 17, 1905. Small dog. While making the preliminary dissection a vessel in the auriculo-ventricular groove was torn; the hæmorrhage that resulted was severe and could not be checked. The hook was, however, inserted. The clamp was closed suddenly with the fingers, but no block resulted. The hook was then withdrawn from the ventricular septum and reinserted. While recording the ventricular contractions alone (it was feared that the animal might die from hæmorrhage if time was taken to arrange the auricular recording apparatus), the clamp was again suddenly closed with the fingers. The ventricle at once came to rest for 22 sec., and then very gradually developed a rapid rate which was independent of that of the auricles. During this time there was no obvious change in the auricular rate. The complete block persisted after unclamping. Then the clamp was again suddenly tightened to see if irritation would cause stoppage of the ventricles. It caused an extra-systole only.

*Autopsy.*—The hook entered the muscular septum on the line of junction of the right and posterior leaflets of the aortic valve and emerged in the right ventricle 5 mm. below the auriculo-ventricular junction and about 5 mm. posterior to the anterior edge of the mesial leaflet of the tricuspid valve.

TABLE XXIII.

| Procedures and Remarks. | | No. of Vs. | Duration in ⅕ Sec. | V. Rate per Min. | No. of As. | Duration in ⅕ Sec. | A. Rate per Min. | $\frac{A}{V}$ | Max. Pres. | Min. |
|---|---|---|---|---|---|---|---|---|---|---|
| Stimulation of vagus. | | 0 | | inhib. | 0 | | inhib. | | | |
| Stim. of accel. | Before. | 28 | 75.2 | 111.7 | 28 | 75.2 | 111.7 | 1 | 119 | [illegible] |
| | During. | 34 | 89.2 | 114.4 | 34 | 89.2 | 114.4 | 1 | 129 | [illegible] |
| | After. | 27 | 70.1 | 115.5 | 27 | 70.1 | 115.5 | 1 | 113 | [illegible] |
| Stim. of accel. | Before. | 20 | 53 | 111.2 | 20 | 53 | 111.2 | 1 | 130 | [illegible] |
| | During. | 43 | 101.3 | 127.3 | 43 | 101.3 | 127.3 | 1 | 146 | [illegible] |
| | After. | 25 | 61.8 | 121.4 | 25 | 61.8 | 121.4 | 1 | 133 | [illegible] |
| Clamped; complete block at once with preliminary slowing of V. Tracing not begun at once. The 1st Vs. recorded=(earlier Vs. much longer) | | 1 | 27.5 | — | 11 | 27.5 | 120 | — | — | [illegible] |
| V. rate then gradually increased until as below. | | | | | | | | | | |
| Stim. of vagus. | Before. | 8 | 53.5 | 44.9 | 21 | 53.5 | 117.8 | 2.6 | 116 | [illegible] |
| | During. | 12 | 86 | 42 | 0 | — | — | — | 93 | [illegible] |
| | After. | 11 | 73.8 | 44.7 | ? | — | — | — | 107 | [illegible] |
| Stim. of accel. | Before. | 13 | 88.3 | 44.2 | 34.7 | 88.3 | 117.9 | 2.7 | 109 | [illegible] |
| | During. | 17 | 115.7 | 44.1 | 50.4 | 115.7 | 130.7 | 2.96 | 126 | [illegible] |
| | After. | 10 | 69.4 | 43.2 | 32 | 69.4 | 138.4 | 3.2 | 105 | [illegible] |
| Stim. of accel. | Before. | 10 | 71.1 | 42.2 | 31.7 | 71.1 | 133.7 | 3.2 | 114 | 108 |
| | During. | 13 | 86.6 | 45 | 38.7 | 86.6 | 134 | 2.98 | 121 | 111 |
| | After. | 11 | 71.5 | 46.2 | 32.2 | 71.5 | 135.1 | 2.93 | 127 | 115 |
| Stim. of accel. | Before. | 9 | 65 | 41.5 | ? | — | — | — | 123 | 113 |
| | During. | 13 | 86.3 | 45.2 | ? | — | — | — | 116 | [illegible] |
| Stim. of accel. | Before. | 13 | 97.2 | 40.1 | 43.6 | 97.2 | 134.6 | 3.4 | 122 | 109 |
| | During. | 12 | 82.3 | 43.7 | 36.8 | 82.3 | 134.2 | 3.07 | 120 | 105 |
| | After. | 10 | 68.2 | 44 | 30.6 | 68.2 | 134.6 | 3.06 | 121 | 110 |
| Stim. of accel. | Before. | 8 | 58 | 41.4 | 25.6 | 58 | 132.4 | 3.2 | 122 | 111 |
| | During. | 12 | 80.7 | 44.6 | 35.9 | 80.7 | 133.2 | 2.97 | 125 | 117 |
| | After. | 12 | 80.2 | 44.9 | 36 | 80.2 | 134.6 | 3.00 | 127 | 118 |
| Stim. of accel. | Before. | 11 | 79.7 | 41.4 | 35.6 | 79.7 | 134 | 3.24 | 113 | 90 |
| | During. | 15 | 101.2 | 44.5 | 45 | 101.2 | 133.6 | 3.00 | 122 | 103 |
| | After. | 10 | 68.2 | 44.4 | 29.8 | 68.2 | 131 | 2.95 | 117 | 98 |
| Stim. of vagus. | Before. | 9 | 65.4 | 41.3 | 29 | 65.4 | 133 | 3.22 | 112 | 91 |
| | During. | 10 | 76.7 | 39.1 | 0 | — | — | — | 98 | 78 |
| Bleeding rapidly from femoral artery. | Before. | 11 | 71 | 46.5 | 30.5 | 71 | 128.9 | 2.77 | 118 | 104 |
| | At beginning. | 10 | 68 | 44.1 | 29 | 68 | 127.9 | 2.9 | 88 | 74 |
| | Near middle. | 11 | 69 | 47.8 | 28.3 | 69 | 123.1 | 2.6 | 44 | 37 |
| | Near end. | 9 | 57.2 | 47.2 | 20 | 57.2 | 104.9 | 2.22 | 18 | 16 |

Exp. 23. March 24, 1905. Small dog. No tracing was made. The hook was inserted without difficulty and without disturbing the normal sequence. The clamp was then carefully tightened with the fingers (the nut was not used). A short period of a 2 : 1 rhythm was followed by stoppage of the ventricles which must have lasted fully 20 sec. The ventricular rate then gradually increased, and when it became constant the block was complete and the rate of the ventricles was about usual for complete heart-block. The auricular rate was not obviously disturbed. Upon unclamping, the block disappeared promptly through a short period of a 2 : 1 rhythm. The clamp was again tightened as before with exactly the same result. The clamp was then loosened,

and while the block was still complete the clamp was suddenly and sharply tightened. The ventricles responded with several extra-systoles; nothing resembling stoppage occurred.

*Autopsy.*—The hook entered the left ventricle at about the middle point of the posterior half of the right aortic leaflet; entered the muscular septum about 5 mm. below and to the left of this point and emerged into the right ventricle at a point slightly anterior to the middle point of the mesial tricuspid leaflet. The tissue included in the clamp was removed for histological examination.

Exp. 24. March 25, 1905. *Effect of electrical stimulation of the tissue included in the clamp.* For this and a few of the subsequent experiments a "clamp electrode" was used. The brass block was replaced by a block of hard rubber. To its lower edge a small plate of platinum was attached. This was united with a fine binding post on the upper edge of the block. All of the fish-hook, excepting 2 or 3 mm. near the point, was coated with a heavy layer of shellac. The upper end of the brass bar into which the hook is soldered was converted into a binding post. These two binding posts were connected with the terminals of a secondary coil. When tested on the tongue it was found that induction shocks passed through only that part of the tongue which was included between the platinum plate and the exposed part of the hook.

The hook was inserted. Upon tightening the clamp very gently with the fingers I obtained a 2 : 1 rhythm, and then released immediately. I stimulated through the clamp with the coil at twenty-seven. The auricles went into fibrillary, or at least very rapid, contractions, and did not recover for 45 min. The ventricles continued to beat, but quite irregularly. When the heart became normal a record was made of the effect of stimulating through the clamp. (Results were neither constant nor clear, and hence are not given here.) The clamp was then tightened until the rhythm became 2 : 1, and later until block became complete. The phenomenon of stoppage of the ventricles was not obtained.

*Autopsy.*—Hook entered through posterior part of right aortic leaflet and passed into the septum about 7 mm. below the highest point of attachment of the valves and on their line of junction. It ran through the septum quite obliquely posteriorly and emerged in the right ventricle about 6 mm. below the auriculo-ventricular junction and a little anterior to the middle point of the mesial tricuspid leaflet.

Exp. 25. March 28, 1905. Very large dog; had previously been used in another experiment in which atropine had been injected intravenously. Strongest stimulation of the vagus was without effect on the heart-rate. No tracings were made. Clamp electrode was placed on the heart and tightened with the fingers, but no block resulted. Clamp was reinserted in the septum and again tightened with fingers; a few beats with 2: 1 rhythm and then complete block resulted. The ventricular rate at first was rather slow and gradually increased to a uniform rate of something between a 2: 1 and 3: 1 rhythm. Although the ventricular rate was at first slow, it was not exactly characteristic of the phenomenon of stoppage. The clamp was then loosened, and while the rhythm was passing through a 2: 1 to normal rhythm, the tissue included in the clamp was stimulated with tetani. With weak currents the rate of both auricles and ventricles was apparently increased. However, this was not very marked even with strong currents—up to twelve. The clamp was again tightened until complete block came on. It was then loosened somewhat, but the block remained complete. Stimulation with increasing strengths of current was again tried. All strengths, but especially the strong, markedly increased the rate of the ventricles. The ventricles fluttered, but the contractions were not fibrillary. The increased rate of the ventricles ceased synchronously with the cessation of stimulation, at least, in most instances. This continued until the coil was at 11.5, when the ventricles went into fibrillary contractions.

*Autopsy.*—The hook entered the aorta near the middle point of the posterior half of the right aortic leaflet. It entered the septum on the line of junction of the right and posterior leaflets about 4 mm. below the uppermost point of attachment. It emerged in the right ventricle about 3 mm. below the auriculo-ventricular junction and about 3 mm. posterior to the anterior edge of the mesial tricuspid leaflet. The tissue included in the clamp was excised for histological examination.

EXP. 26. March 30, 1905. Medium-sized dog. The clamp electrode was adjusted on heart and tightened with fingers; no block resulted. Hook was reinserted in septum and clamp again tightened with fingers; complete block came on at once preceded by the phenonemon of stoppage. When hook was unclamped, normal sequence returned. I now began to make tracings. At various times the tissue in the clamp was stimulated with tetani of increasing strength. The results were not constant and have not been included in the analysis given below.

*Analysis of Tracings of Exp. 26.*—Clamp tightened: complete block came on at once, preceded by marked preliminary slowing of the ventricles. 1st ventricular cycle lasted 14.1 sec., and in it blood-pressure fell to 21 mm. Hg. During this ventricular cycle the auricular rate was 145.7 per min. (The auricular rate had been 154.3 before clamping.) Ventricular rate then gradually increased and when it became constant it was 29.7.

Hook unclamped: 2 : 1 rhythm came on and persisted.

Atropine (4 mgr.) administered intravenously.

Stimulation of vagus: no slowing of auricle or ventricle.

Hook clamped (rhythm still 2 : 1): 1stventricular systole somewhat longer than others, but I cannot be certain that complete block was obtained because ventricular rhythm was not regular.

Hook unclamped: 2 : 1 rhythm.

Stimulation of vagus: no slowing of auricle or ventricle.

Hook clamped: 3 : 1 rhythm (apparently—auricular tracing not distinct).

Hook unclamped: auricles too weak to record.

*Autopsy.*—The hook entered the aorta exactly as in the preceding experiment. It entered the septum on the line of junction of the two leaflets and 7 mm. below their uppermost line of attachment. It emerged in the right ventricle about 5 mm. below the auriculo-ventricular junction and about 5 or 6 mm. posterior to the anterior edge of the mesial tricuspid leaflet.

EXP. 27. March 31, 1905. Large dog. Hook inserted and clamp tightened with fingers—no block. Hook withdrawn from septum and reinserted—complete block without stoppage. Tracings were then made with the Hürthle manometer. In this experiment, upon tightening the clamp complete block never came on at once, indeed there was an unusually wide margin between the normal sequence and the various stages of block.

*Autopsy.*—The hook entered the aorta rather low and somewhat anterior to the line of junction of the right and posterior aortic leaflets. It entered the septum 2 or 3 mm. posterior to this line and about 8 mm. below the uppermost line of attachment of the valves. It entered the right ventricle 4 mm. below the auriculo-ventricular junction and about 6 mm. posterior to the anterior edge of the mesial tricuspid leaflet.

*Results.*—The clamp electrode was used in this experiment and the tissue included in it was stimulated with various strengths of current. The results obtained were not consistent; they have not received a satisfactory interpretation, and therefore will not be presented now.

While unclamping I obtained one ventricular silence in 17, 20, 24, and 23 auricular cycles.

While clamping (with rapidly revolving drum) 1: 1 rhythm passed into alternation of 2–1: 1 with 1–2: 1. Then a 3: 1 rhythm developed and continued through a long period of clamping. It was with great difficulty that complete block was obtained.

A record was then made upon the rapidly revolving drum of the recovery from this block. The following were obtained: 3: 1 and 2: 1 rhythms; one ventricular silence in ten, nine, eight, seven, six, five, four, and three auricular beats.

Careful measurement of these tracings shows that the ventricular systole which follows each silence comes sooner after its corresponding auricular systole than the other ventricular systoles. In the succeeding cycles the ventricular systoles follow their auricular systoles later and later until another ventricular systole is dropped. The lengthening of the intersystolic interval is most rapid in the cycles which immediately precede the ventricular silences.

Exp. 28. April 4, 1905. Small dog. Clamp was adjusted on heart. When it was tightened with the fingers the sequence of the heart-beat did not change. The hook was withdrawn from the septum and reinserted. Now when the clamp was tightened with the fingers complete block resulted without a tendency toward stoppage. Stimulation of the vagus inhibited the auricles, but not the ventricles. After unclamping the sequence slowly returned to normal. The hook was again withdrawn from the septum and reinserted farther anteriorly. Now upon tightening the clamp with the fingers, complete block came on with some, although not marked, preliminary slowing of the ventricles. When the hook was unclamped, normal sequence returned. Atropine, 2 mgr., was injected directly into the heart. Stimulation of the vagus caused no inhibition. The clamp was tightened with the fingers and complete block without any obvious tendency toward stoppage resulted. When the hook was unclamped, sequence became normal. Clamp was tightened with results same as before.

*Autopsy.*—The hook entered the aorta between the orifice of the right coronary artery and the posterior edge of the right leaflet. It entered the muscular septum about 5 mm. below the highest point of union of the right and posterior aortic leaflets. The point was found in the right ventricle about 5 mm. below the auriculo-ventricular junction and a few millimeters posterior to the anterior edge of the mesial tricuspid leaflet.

The first position of the hook in the septum was marked by thrombi adherent to the torn endocardium. These were somewhat posterior to the first position and about 1 cm. below the uppermost point of attachment of the aortic leaflets.

Exp. 29. April 5, 1905. Small dog. The clamp was adjusted on the heart and tightened with the fingers: complete block was obtained with intermediate stages and without preliminary stoppage. The clamping was repeated several times with similar results.

The hook was withdrawn and reinserted into the septum. Upon tightening the clamp with the fingers I obtained complete block with short intermediate stages and with a short preliminary stoppage. The complete block did not disappear for about an hour after unclamping.

*Autopsy.*—The hook slipped out of the septum while excising the heart. From the position of the holes in the septum it was judged that the hook had passed through the septum about in the line of junction of the right and posterior aortic leaflets and had entered the right ventricle from 2 to 3 mm. below the auriculo-ventricular junction.

Exp. 30. April 12, 1905. Large dog. The clamp was put in place and tightened, but no block resulted. The hook was then reinserted in the septum. Now when it was tightened with the fingers there were several extra-systoles followed by complete stoppage of the ventricles for twenty-nine auricular beats. The ventricular rate gradually increased until there was about one ventricular systole for two auricular systoles and although the clamp was immediately opened the block remained complete for about a half-hour. During this time stimulation of the vagus inhibited the auricles, but not the ventricles. Then an inconstant 2 : 1 rhythm developed. Now stimulation of the vagus inhibited the auricles as before, the ventricles for a short time (about 4 sec.). While the heart was recovering from inhibition it frequently passed through a 1 : 1 rhythm.

The 2 : 1 rhythm was maintained for about 30 min. and then gradually returned to complete block, from which the heart did not recover. After the lapse of another hour the clamp was abruptly tightened with the fingers. Extra contractions of the ventricles were obtained, but no stoppage.

*Autopsy.*—While excising the heart the hook slipped out of the septum. The position it had occupied was marked by hæmorrhage into the heart muscle. As viewed from the right side the hæmorrhage was at about the middle of the posterior edge of the membranous septum and extended about 3 mm. into the muscular tissue. No hole could be found in the septum corresponding with the first position of the clamp.

Exp. 31. April 15, 1905. Small dog. Two attempts to produce block failed. The hook was then entirely withdrawn from the heart and reinserted anew. Now upon tightening the clamp with the fingers I obtained partial block for a few beats and then complete block without marked preliminary slowing of the ventricles. When the hook was released, at once normal sequence returned. I tried to determine the weakest stimulation of the vagus which would inhibit the ventricles in the different rhythms, but these could not be maintained long enough to allow of satisfactory determinations.

I then used strong stimulation of the vagus and determined the duration of inhibition in the different rhythms.

Upon unclamping complete block was obtained preceded by a few cycles of 2:1 rhythm. Then I unclamped hook.

| Position of coil. | Rhythm. | Duration of inhibition of ventricles in seconds. |
|---|---|---|
| 11 | 2:1 | 5. |
| 11 | 1:1 | 9.2 |
| 11 | 1:1 | 19.4 |
| 11 | 1:1 | 20.4 |

I tightened clamp carefully with screw: complete block without intermediate stages and with a rather long preliminary stoppage of ventricles followed. I unclamped at once: recovery was very rapid. The following were obtained within a few seconds after recovery from complete block:

| Position of coil. | Rhythm. | Duration of inhibition. |
|---|---|---|
| 11 | 1:1 | 3. |
| 11 | 1:1 | 4.2 |
| 11 | 1:1 | 4.4 |
| 11 | 1:1 | 4.4 |

Upon clamping I got complete block at once with preliminary stoppage of the ventricles, the longest ventricular cycle lasting twenty-one auricular systoles (auricular rate = 20 in 16 sec.). Upon unclamping normal sequence returned very quickly: immediate stimulation of vagus with coil at four inhibited the ventricles 3.8 sec. Then:

| Position of coil. | Rhythm. | Duration of inhibition. |
|---|---|---|
| 8 | 1:1 | 4.8 |
| 8 | 1:1 | 4.2 |

Upon clamping I got complete block at once with the following time relations:

| No. of ventricular systole: . . . . | 1st. | 2d. | 3d. |
|---|---|---|---|
| Duration in auricular systoles. | 4 | 23 | longer, and then ventricular rate gradually increased. |

Heart unclamped, during recovery:

| Position of coil. | Rhythm. | Duration of inhibition. |
|---|---|---|
| 8 | 2:1 | 4.2 |

Upon clamping I got complete block with preliminary stoppage of ventricles as follows:

| No. of ventricular systole: . . . . . . | 1st. | 2d. | 3d. | 4th. |
|---|---|---|---|---|
| Duration in auricular systoles. . | 5 | 22 | 9 | 4 |

I unclamped immediately and before complete block had passed away clamped sharply and suddenly: nothing obvious happened. Upon unclamping the heart did not recover from complete block.

Every time the clamp was carefully tightened, particularly toward the close of the experiment, the following events were noted: After screwing down to a certain point it could be seen that the intersystolic interval increased in length. At this time the contractions of the ventricles became peculiar: although the rhythm and rate of contractions remained unchanged, the ventricular muscle showed a curious twisting or writhing motion such as is sometimes seen before the ventricles begin to fibrillate. When this stage was reached the nut was driven down about one half a turn at a time with long pauses between. It was always immediately after the completion of one of these half-turns that the complete block suddenly came on.

*Autopsy.*—The hook had entered the aorta near the posterior edge of the right leaflet and entered the septum below the line of junction of the right and posterior leaflets, and 8 mm. below their uppermost point of attachment. The point emerged into the right ventricle on a line perpendicular to the middle point of the posterior edge of the membranous septum and about 3 mm. from this edge.

No marks could be seen in the septum of the earlier positions of the clamp and it is therefore probable that it had not entered the septum at all.

## EXPLANATION OF PLATES I–IV.

Fig. 20.—Showing different stages of recovery from a 2 : 1 rhythm. Upper tracing = auricular beats (down strokes). Lower tracing = pressure in the central end of the carotid artery with the Hürthle manometer. Time = ⅕ sec.

A = 1 ventricular silence in 2 auricular cycles.
B = 1 " " 3 " " .
C = 1 " " 4 " " .
D = 1 " " 5 " " .
E = 1 " " 6 and 7 auricular cycles.
F = 1 " " 8, 9, and 10 auricular cycles.
G = Occasional ventricular silences and the return to normal.

Fig. 21.—Showing the usual effect of clamping and unclamping. A = normal. B = 2 : 1 rhythm. C = 3 : 1 rhythm. D = complete block. E = 3 : 1 rhythm. F = 2 : 1 rhythm. G = normal.

Fig. 22.—Cardiogram of complete heart-block in the dog.

Fig. 23.—Showing the effect of stimulation of the vagus in various stages of heart-block. Upper tracing = auricular contractions. Lower = pressure in the central end of the carotid artery with a mercury manometer. Time on base line = ⅕ sec. A = during normal sequence. B = during 2 : 1 rhythm. C = during complete block.

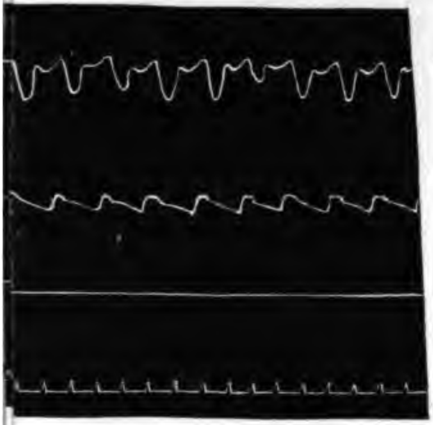
Fig. 24.—Showing the effect of stimulation of the vagus during normal sequence (A and C) and during complete block (B): otherwise same as Fig. 23.

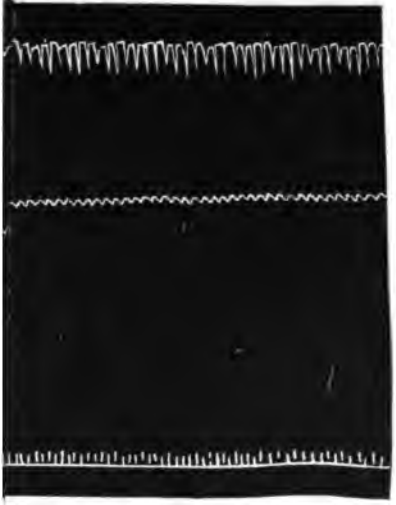
Fig. 25.—Showing the effect on the pulse-pressure (ventricular output) of an escape auricular beat during vagus inhibition. The heart-block is complete. Otherwise same as Fig. 23.

Fig. 26.—Showing one of the effects of gradually clamping the region of the auriculo-ventricular bundle—ventricular stoppage associated with the sudden development of complete heart-block. Otherwise same as Fig. 23.

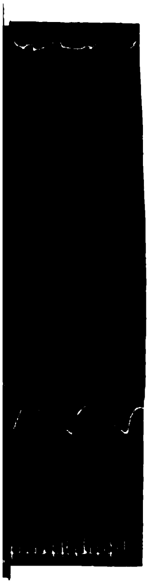
Fig. 27.—Jugular sphygmogram (upper) and cardiogram (lower) showing a regularly recurring ventricular extra-systole.

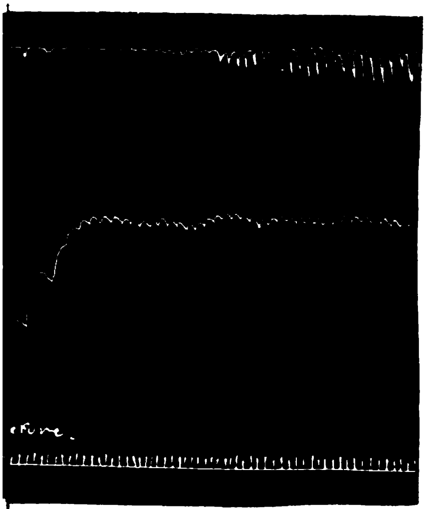
PLATE II.



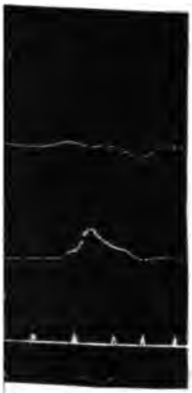


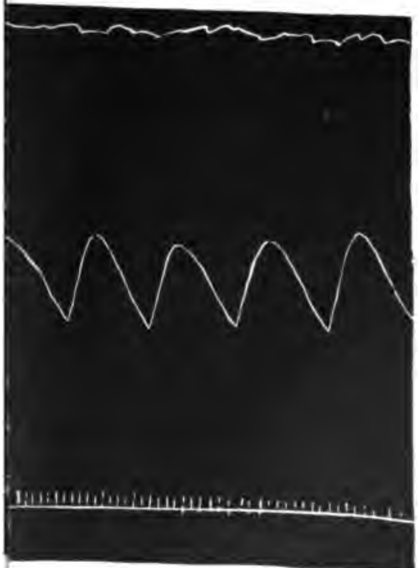
# PLATE III.





# PLATE IV.





# THE EFFECT OF CHANGES IN TEMPERATURE UPON THE VISCOSITY OF THE "LIVING" BLOOD.[1]

BY R. BURTON-OPITZ.

*(From the Physiological Laboratory of Columbia University, at the College of Physicians and Surgeons, New York.)*

It has been shown in an earlier paper of this series[2] that the viscosity of defibrinated blood can be changed by varying the temperature. While a rise in the temperature produces a decrease, a fall in the temperature causes an increase of the "molecular" friction of the blood. The question now arises, does the viscosity of the "living" blood vary in a similar manner, or better, is it possible to alter the viscosity of the circulating blood by changing the temperature of the surrounding medium?

To solve this question I performed a series of experiments upon dogs in accordance with the method described by Hürthle.[3] By means of this method it is possible to obtain a number of factors which are made use of subsequently in determining K, the coefficient of the viscosity of the blood. The factors involved in this calculation are the quantity of blood collected, during a definite period, the blood pressure, the specific gravity of the blood, and the length and internal diameter of the capillary-tube. The coefficients expressing the viscosity of the blood, are then compared with the coefficient of distilled water at 37° C., which, according to Poiseuille, possesses the value 4700. Hence, the viscosity of the blood is denoted in terms of multiples of the viscosity of distilled water at 37° C.

Only one capillary-tube was used in these experiments; its internal diameter measured 0.6636 mm. and its length 213.0 mm.

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[1] This study was conducted under a grant from the Rockefeller Institute for Medical Research.

[2] Burton-Opitz, R., *Archiv für die ges. Physiol.*, 1900, lxxxii, 468.

[3] Hürthle, K., *Archiv für die ges. Physiol.*, 1900, lxxxii, 415.

The accuracy of the tube was tested by a series of preliminary experiments with distilled water.

The experiments were performed upon dogs during uniform light ether narcosis, no morphine having been administered beforehand. I tested the effect of warm and cold water baths and of hot dry air.

### A.—THE EFFECT OF WARM AND COLD BATHS.

Six experiments in all were made, three with warm (43° C.) and three with cold water (23° C.). After securing the animal in a bath-tub, the normal viscosity of the blood was tested by three determinations at room temperature. The tub was then filled with warm or cold water. As the carotids were needed in experimentation, the ventral half of the neck and head were allowed to project above the surface of the water. Three other determinations of the viscosity were made fifteen, thirty, and forty-five minutes after the beginning of the bath.

The results of these experiments are compiled in Tables I and II; the first contains the experiments with warm, and the second, those with cold water. Each experiment is again subdivided into two parts, showing, on the one hand, the determinations of the normal viscosity, and, on the other, the determinations of the viscosity as influenced by the warm or cold bath. Among other details the tables also give the rectal temperature of the animal at the beginning and end of the experiment and the specific gravity of the blood. The latter factor was determined several times in the course of each experiment. Small pycnometers were used for this purpose.

These experiments prove conclusively that the viscosity of the blood reacts very sharply toward changes in the temperature of the surrounding medium. We observe that, in the case of the warm water, the coefficients become numerically larger than normal, while, in the case of the cold water, the coefficients become smaller. This implies that *the viscosity is decreased by warm and increased by cold water baths.* Let us take, for example, Experiment 2 of Table I. The viscosity of the blood (K = 862) is in this case 5.4 times as great as that of distilled

## TABLE I.

### THE EFFECT OF WARM WATER BATHS.

| aber of Experiment d Weight of Dog. | | Determination Number. | Temperature °C. | | Time Elapsed since Beginning of Bath. Min. | Specific Gravity of the Blood. | Quantity Escaped from Capillary-Tube. Mgr. | Time. Sec. | Pressure Mm. Hg. | Viscosity Co-efficient, K. | Mean Value of K. | Greatest Difference. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | of water. | of animal, rectal. | | | | | | | | |
| I. kg. | Normal..... | 1 | — | 37.2 | — | 1.05865 | 1688.3 | 13.04 | 168.60 | 796.71 | 788.47 | 74 43 |
| | | 2 | — | " | — | " | 1789.7 | 14.43 | 163.40 | 787.50 | | |
| | | 3 | — | 37.0 | — | " | 1530.8 | 12.05 | 169.20 | 781.21 | | |
| | During Bath | 4 | 43.0 | — | 15 | 1.05802 | 1667.4 | 13.47 | 153.92 | 834.88 | 846.09 | |
| | | 5 | " | — | 30 | 1.05690 | 2039.2 | 15.08 | 168.00 | 840.50 | | |
| | | 6 | " | 41.3 | 45 | " | 2417.3 | 14.96 | 194.60 | 862.90 | | |
| II. kg. | Normal..... | 1 | — | 37.4 | — | 1.05355 | 1594.9 | 11.48 | 164.40 | 881.00 | 862.81 | 119.98 |
| | | 2 | — | " | — | " | 1687.3 | 12.44 | 165.60 | 853.87 | | |
| | | 3 | — | 37.1 | — | " | 1768.9 | 12.62 | 171.20 | 853.56 | | |
| | During Bath | 4 | 43.5 | — | 15 | 1.05260 | 1573.6 | 11.37 | 155.80 | 926.92 | 954.61 | |
| | | 5 | " | — | 30 | 1.05202 | 1886.5 | 12.20 | 169.20 | 954.14 | | |
| | | 6 | " | 42.0 | 45 | " | 2346.5 | 14.90 | 167.30 | 982.79 | | |
| . III. kg. | Normal..... | 1 | — | 37.5 | — | 1.0500 | 2004.2 | 13.28 | 151.90 | 1039.32 | 1038.09 | 101.39 |
| | | 2 | — | " | — | " | 1782.1 | 11.89 | 149.60 | 1048.00 | | |
| | | 3 | — | 37.2 | — | " | 1663.1 | 12.32 | 137.50 | 1026.95 | | |
| | During Bath | 4 | 42.0 | — | 15 | — | 1782.9 | 10.39 | 159.60 | 1126.48 | 1134.11 | |
| | | 5 | " | — | 30 | 1.04832 | 1768.9 | 10.78 | 151.30 | 1136.38 | | |
| | | 6 | " | 39.8 | 45 | " | 2038.7 | 12.26 | 152.90 | 1139.48 | | |

## TABLE II.

### THE EFFECT OF COLD WATER BATHS.

| umber of Experiment and Weight of Dog. | | Determination Number. | Temperature °C. | | Time Elapsed since Beginning of Bath. Min. | Specific Gravity of Blood. | Quantity Escaped from Capillary-Tube. Mgr. | Time. Sec. | Pressure Mm. Hg. | Viscosity Co-efficient, K. | Mean Value of K. | Greatest Difference. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | of water. | of animal. | | | | | | | | |
| p. I. o kg. | Normal..... | 1 | — | 38.0 | — | 1.06290 | 1183.4 | 9.65 | 170.60 | 742.82 | 721.31 | 87.46 |
| | | 2 | — | " | — | " | 1536.9 | 10.76 | 204.20 | 722.80 | | |
| | | 3 | — | 37.9 | — | " | 1592.4 | 12.19 | 193.30 | 698.33 | | |
| | During Bath | 4 | 22.5 | — | 15 | 1.06495 | 1761.3 | 13.77 | 198.00 | 666.27 | 647.53 | |
| | | 5 | " | — | 30 | 1.06570 | 1907.5 | 15.33 | 199.60 | 642.49 | | |
| | | 6 | " | 32.4 | 45 | " | 2043.7 | 18.38 | 180.80 | 633.85 | | |
| p. II. 2 kg. | Normal..... | 1 | — | 36.9 | — | 1.06030 | 1620.7 | 12.28 | 163.50 | 836.20 | 832.00 | 114.63 |
| | | 2 | — | " | — | " | 2341.0 | 14.80 | 199.00 | 823.40 | | |
| | | 3 | — | 36.9 | — | " | 1860.3 | 13.69 | 168.30 | 836.40 | | |
| | During Bath | 4 | 23.0 | — | 15 | 1.06065 | 1859.0 | 16.08 | 157.50 | 760.10 | 741.72 | |
| | | 5 | " | — | 30 | 1.06238 | 1806.1 | 14.76 | 169.20 | 747.70 | | |
| | | 6 | " | 33.0 | 45 | " | 2069.3 | 17.42 | 171.20 | 717.37 | | |
| p. III. 12 kg. | Normal..... | 1 | — | 37.0 | — | 1.05236 | 1367.7 | 12.90 | 113.30 | 976.70 | 978.02 | 129.17 |
| | | 2 | — | " | — | " | 1742.1 | 13.62 | 136.50 | 978.01 | | |
| | | 3 | — | 37.0 | — | " | 1871.7 | 13.92 | 143.30 | 979.36 | | |
| | During Bath | 4 | 22.0 | — | 15 | 1.05375 | 1761.9 | 14.20 | 135.60 | 953.80 | 898.32 | |
| | | 5 | " | — | 30 | " | 2025.7 | 17.17 | 136.00 | 904.22 | | |
| | | 6 | " | — | 45 | " | 2382.6 | 17.61 | 159.10 | 886.41 | | |
| | | 7 | " | 33.5 | 60 | " | 1198.3 | 11.17 | 131.70 | 848.85 | | |

water at 37° C. (K=4700). Under the influence of the warm water bath, the viscosity was reduced in the course of forty-five minutes (K=982) to only 4.7 times as great as that of distilled water at 37° C. Equally pronounced effects were obtained with cold water. In Experiment 3, Table II, the viscosity of the blood was increased in the course of forty-five minutes from 4.8 to 5.5 times as great as that of distilled water at 37° C. Moreover, when a comparison is made between the time and the total increase or decrease in the viscosity, it is seen that by far the greatest change takes place during the first ten to fifteen minutes after the beginning of the bath.

The specific gravity of the blood pursued in these experiments a course parallel to the viscosity. Warm water baths produced a decrease and cold water baths an increase in the specific gravity. The changes were very pronounced in both cases.

### B.—THE EFFECT OF HOT AIR BATHS.

The method followed in the three experiments of this group was similar to that described in an earlier paragraph of this paper. The animal was placed in a glass case, through which a constant stream of dry hot air could be passed. As the carotids were used in experimentation, it was necessary to keep the head and neck of the animal outside of the case. As in the preceding six experiments, the animal was allowed to pant freely.

The normal viscosity of the blood having been determined several times, the temperature of the air in the case was gradually raised to about 60° C. Three determinations were then made, fifteen, thirty, and forty-five minutes afterward.

The results of the experiments are given in Table III. Each experiment is divided into two parts, showing the viscosity of the blood before, as well as during the air bath. The table also contains the temperature of the animal at different periods of the experiment, the temperature of the air, and the specific gravity of the blood.

A comparison of the coefficients proves that hot air baths do not render the blood less viscous, as one might suppose, but more

## TABLE III.

### EFFECT OF HOT AIR BATH.

| mber of Experiment nd Weight of Dog. | | Determination Number. | Temperature ° C. | | Time Elapsed since Beginning of Bath. Min. | Specific Gravity of Blood. | Quantity Escaped from Capillary-Tube. Mgr. | Time. Sec. | Pressure. Mm. Hg. | Viscosity Coefficient, K. | Mean Value of K. | Greatest Difference. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | of air. | of animal. | | | | | | | | |
| . I. kg. | Normal..... | 1 | — | 37.2 | — | 1.06118 | 1475.3 | 12.17 | 160.70 | 780.80 | 769.07 | 22.07 |
| | | 2 | — | " | — | " | 1492.2 | 12.87 | 156.70 | 765.85 | | |
| | | 3 | — | 37.1 | — | " | 1588.3 | 13.22 | 163.50 | 760.58 | | |
| | During Bath | 4 | 65.0 | — | 15 | 1.06222 | 1448.2 | 12.67 | 155.80 | 758.58 | 751.74 | |
| | | 5 | " | — | 30 | " | 1335.7 | 12.61 | 146.10 | 749.66 | | |
| | | 6 | " | 41.0 | 45 | 1.06480 | 1119.5 | 11.40 | 135.60 | 747.00 | | |
| ). II. ) kg. | Normal..... | 1 | — | 37.0 | — | 1.05338 | 1466.3 | 12.63 | 127.90 | 946.46 | 930.51 | 42.41 |
| | | 2 | — | " | — | " | 1481.7 | 13.64 | 123.00 | 920.88 | | |
| | | 3 | — | 36.8 | — | " | 1574.9 | 13.28 | 133.80 | 924.19 | | |
| | During Bath | 4 | 70.0 | — | 15 | 1.05520 | 1626.5 | 12.26 | 153.70 | 898.45 | 891.85 | |
| | | 5 | " | — | 30 | " | 1530.8 | 13.15 | 137.30 | 889.00 | | |
| | | 6 | " | 42.2 | 45 | " | 1053.6 | 12.77 | 96.70 | 888.10 | | |
| p. III. ) kg. | Normal..... | 1 | — | 37.2 | — | 1.05435 | 2017.6 | 14.28 | 152.90 | 962.64 | 975.44 | 54.89 |
| | | 2 | — | " | — | " | 1950.5 | 14.71 | 142.00 | 972.75 | | |
| | | 3 | — | 37.0 | — | " | 2293.7 | 13.17 | 183.10 | 990.95 | | |
| | During Bath | 4 | 60.0 | — | 15 | 1.05500 | 2056.2 | 13.27 | 171.10 | 942.81 | 931.72 | |
| | | 5 | " | — | 30 | " | 1802.6 | 13.61 | 148.00 | 931.81 | | |
| | | 6 | " | 40.5 | 45 | " | 1811.5 | 14.53 | 141.00 | 920.55 | | |

so. Hence, the hot air and warm water produce directly antagonistic results. It seems, however, that the former is less powerful, because the increase in the viscosity produced by a constant exposure of the body to air of about 60° C. did not amount to more than fifty-five points in any of the experiments. For example, in Experiment 3, of Table III, the normal viscosity was 4.8 times as great as that of distilled water at 37° C. Under the influence of the hot air it became, in the course of forty-five minutes, 5.1 times as great. We will remember that the differences obtained with warm and cold water were much more marked and rapid.

The specific gravity of the blood became greater during the hot air bath. The viscosity and specific gravity preserved, therefore, a direct relationship with one another.

The coefficients for the normal blood of the dog vary in the present nine experiments between 1038 and 721, the average being 877. The viscosity of the dog's blood is therefore 5.3 times as great as that of distilled water at 37° C.

# EXPERIMENTAL CIRRHOSIS OF THE LIVER.*

By RICHARD M. PEARCE, M.D.

(*From the Bender Laboratory, Albany, N.Y.*)

PLATE V.

The experimental studies upon which this communication is based were suggested by an investigation [1] of the necroses occurring in the liver of the dog as the result of the intravenous injection of hæmolytic immune sera. These necrotic lesions, which appear to have a very definite relation to thrombi composed of fused red blood corpuscles, vary in position and extent according to the amount of serum administered. Small doses cause focal lesions more or less isolated and irregularly distributed; large doses produce a diffuse necrosis which spares only the tissue about the larger portal spaces. The uniformity and extent of this latter lesion suggested the importance of studying the repair process which naturally follows it in those animals surviving the acute toxic effects of the serum. It was evident that the extent of the injury was such as to preclude complete repair by regeneration of liver cells alone. If, then, the defect were to be repaired by connective-tissue proliferation the resulting histological picture would, except for a difference in the distribution of the new tissue, closely resemble cirrhosis in man.

Experiments based on this method have not, heretofore, been made. Joannovics,[2] alone of the few investigators who have studied the lesions of the liver due to hæmolytic sera, has described repair about foci of necrosis. Flexner,[3] in 1894, while studying the lesions occurring in the organs of the rabbit after the injection of serum of the dog, observed a well-marked cirrhosis due apparently to repair about multiple necroses. The lesion was found, however, but once, and could not be repro-

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* This study has been conducted under a grant from the Rockefeller Institute for Medical Research. Read before the American Association of Pathologists and Bacteriologists in Chicago, April 19, 1905.

duced in other animals. The literature contains, as far as I am aware, no other references to similar lesions due to serum injections.

*Methods.*—Dogs were injected either in the smaller branches of the femoral vein or in the abdominal cavity with serum obtained from rabbits which had received repeated injections of the red blood corpuscles of the dog. The dose usually employed was one cubic centimeter of serum to from 500 to 1000 grammes of body weight. The majority of the animals died within forty-eight hours; those which survived were killed at intervals varying from forty-eight hours to thirty-six days.

*Character of the Primary Necrosis.*—The necrotic lesions in the liver vary according to the dose of the serum. As the etiology and the general character of the focal necroses have been discussed elsewhere, only the more extensive form of necrosis, which through an equally widespread process of repair leads to cirrhosis, will be considered at this time. The macroscopic appearance of the liver in animals dying within a few minutes or hours after the injection of serum is that of intense congestion. Microscopically, the vessels are found to be distended by closely massed red blood corpuscles. The coalescence of red cells is very evident in the portal veins, while in the capillaries the occlusion leads to a distension so great that the columns of liver cells are more or less obscured. This congestion is less evident about the larger portal spaces. After twenty-four to forty-eight hours, the liver presents a uniformly mottled appearance; fine irregular yellowish brown or grayish yellow non-elevated areas being sharply separated from deeply congested or occasionally even hæmorrhagic areas. On section the brownish portions have a distinct hyaline appearance. The superficial portions of the liver are uniformly more extensively involved than are the deeper portions, though in some of the smaller lobes the necrosis may be quite general. Histologically, the necrosis, which is hyaline in character, involves all portions of the liver tissue except circular areas of varying size in the immediate neighborhood of the larger portal spaces (see Fig. 1).

In the necrotic areas the destruction, as far as the hepatic cells are concerned, is uniform and complete; the cells of the capillaries may, however, persist. A narrow ring of liver cells with vacuolated protoplasm and pyknotic nuclei separates, as a rule, the necrotic from the normal tissue. Leucocytic infiltration may or may not be present. The capillaries of the necrotic tissue are widely dilated and tightly packed with swollen and distorted red blood corpuscles which may occur in masses or may retain distinct outlines. In the congested portal veins, on the other hand, the fusion is quite constant, the plugs for the greater part having a distinctly hyaline appearance. The perivascular spaces of the capillaries are distended with serum, and the bile passages of all sizes are, as a rule, dilated and engorged with bile.

The cause of the necrosis is an obstructive congestion of the capillaries and the smaller branches of the portal vein by fused masses of red cells. To the effect of this obstruction, essentially a thrombosis, is added the pressure exerted by the perivascular œdema and the over-filled bile capillaries. In parts of the liver midway between the larger portal spaces these factors are sufficient to overcome the pressure in the capillaries from the hepatic artery, and necrosis results; the cells in the neighborhood of the larger portal spaces, on the other hand, are preserved through a better blood supply dependent, presumably, upon the greater arterial pressure in this region.

A review of the literature of infarction of the liver in man and of experimental infarction shows no condition analogous to that here described; nor are any of the theories put forth concerning the etiology of infarction applicable to it. A possible exception is the widespread hæmorrhagic infarction produced by Wooldridge[4] as the result of injecting into the jugular vein of the dog a complex proteid substance derived from the thymus and other glandular organs. The infarctions were associated with thrombi in the branches of the portal vein, and in this regard, as well as in the character of the necrosis, Wooldridge's lesion resembles that just described. Moreover, in animals which survived fourteen days, he observed scattered foci of repair resembling early cirrhosis. He does not consider the thrombosis alone to be sufficient cause for the necrosis, but believes that an important adjuvant is some change in the chemical composition of the blood caused by the proteid substance injected.

*The Reparative Process.**—Only fifteen of forty-three animals survived the acute effects of the serum a sufficient length of time to allow repair of the liver lesion to take place. The liver of each, however, shows some stage of repair, and as various periods of from thirty-eight hours to thirty-six days are represented, it has been possible to study all stages of the development of the cirrhotic lesion.

At thirty hours the first and only evidence of repair is karyokinesis of the liver cells. This, however, is not conspicuous and the mitotic figures are found only after prolonged search. They occur, uniformly, not at the edge of the necrotic tissue but in liver cells two or three rows removed. Mitotic figures have never been seen earlier than thirty-eight hours, and at this period in but one animal.

The period from forty-eight to sixty hours is represented by five animals. The character of the repair is the same in all, but some difference in degree is evident. The most striking feature is the proliferation of the endothelial cells. These surround the necrotic tissue and penetrate it from every side. Similar cells, corresponding to the remains of the capillaries, may be seen in some places within the necrotic areas. Many of these cells have irregular nuclei suggestive of mitosis, but a definite karyokinetic figure, in a cell surely endothelial, was found at this stage but once; this was in the liver of an animal killed exactly forty-eight hours after injection. The endothelial cells show a tendency to surround small fragments of necrotic liver cells, and occasionally they contain one or two red blood corpuscles. Leucocytic infiltration occurs to a very slight extent.

By the end of the fourth day the proliferation has advanced so rapidly that the bulk of the necrotic tissue has been replaced.

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* Only a general outline of the reparative process will be given at this time; the details of the finer changes and their interpretation will be presented in a future communication on the repair of liver tissue.

Within the necrotic areas the bands of new tissue are more definite. Mitotic figures in liver cells are so numerous that three or four may be found in one field of a one-twelfth lens. A few lymphoid cells are seen in the portal spaces.

The picture at the fifth day differs only in the extent of the process. The new tissue has assumed all the characteristics of granulation tissue and contains giant cells which surround fragments of hyaline material. The first suggestion of intercellular fibrillæ is seen at this stage.

The later stages, from the eleventh to the thirty-sixth day, illustrate the gradual transformation of this young granulation tissue into a very definite connective tissue. At the eleventh day the new blood-vessels are very prominent and the fibrillated character of the connective tissue well marked. All stages after the fourth day show a few scattered lymphoid cells, but this has not been a prominent feature except in one animal, killed on the twenty-fifth day, in which the tissues of the portal spaces are filled with lymphoid and plasma cells.

The oldest as well as the most typical lesion was that found in an animal killed on the thirty-sixth day (see Fig. 2). Macroscopically, the liver was much firmer than normal, had a finely granular surface, and was deeply bile stained. The capsule showed irregular areas of thickening and was mottled by an ill-defined congestion. On section, a distinct pseudo-lobulation was evident, definite islands of brownish yellow liver tissue being marked off by a fine grayish network of newly formed tissue. Histological examination shows broad bands of connective tissue entirely replacing the necrotic areas and forming a uniformly arranged network separating the surviving islands of liver tissue about the larger portal spaces. The new tissue is distinctly fibrous and contains many newly formed blood-vessels and bile ducts; it stains deeply by Mallory's method for connective tissue. The formation of new liver cells is still demonstrable. Fragments of necrotic hyaline cells, not infrequently partially calcified, are found in the midst of the new connective tissue. Large irregular multinucleated masses of protoplasm surround these and are apparently active in englobing and removing them.

These multinucleated cells, essentially foreign body giant cells, are derived in part from endothelial cells and in part from liver cells. Associated with them are numerous multinucleated liver cells without inclusions. Lymphoid and plasma cells are present, but only in small numbers.

*Discussion.*—The final stage of the lesion herein described, constituting as it does a chronic interstitial hepatitis of diffuse but uniform distribution, may justly be termed an experimental cirrhosis. No close analogy, however, can be drawn between it and human cirrhosis, for, with the possible exception of the so-called central cirrhosis associated with chronic passive congestion, a form of interstitial hepatitis with similar distribution of the new tissue does not occur in man. In this connection Bostroem's [5] recent discussion of liver cirrhosis as a repair process in chronic passive congestion is of especial interest. As the result of a study of material from man he reaches the conclusion that the congestion causes destruction of liver cells, not necessarily by pressure of the widened capillaries, but through nutritive and functional disturbances. With these conditions are associated rupture of the capillary wall and the escape of red cells into the perivascular lymph spaces. Thrombosis also is not an infrequent occurrence and has a close relation to the destruction of liver cells. The repair, which is most active in the region of the portal spaces, results in a very definite cirrhosis. When one considers that the necrosis in my experimental lesion is preceded by an intense congestion associated with all the etiological factors described by Bostroem, the analogy of the repair lesion to the cirrhosis which he describes becomes very apparent. Comparisons as to the etiology of cirrhosis in general are not justifiable, for we know of no toxic substances associated with abnormal conditions in man which are capable of causing the widespread necrosis seen in these experimental lesions. The only condition comparable is acute yellow atrophy with which the early necrotic and reparative lesions of the second to the sixth day have much in common.

On the other hand, however, these experimental lesions demonstrate that a cirrhosis may follow extensive primary destructive lesions, and thus support the theory of Kretz [6] that cirrhosis is essentially a reparative process. Kretz, who bases his conclusions on an extensive study of the disease in man, believes cirrhosis to be the result of successive processes of repair following repeated focal injuries of the liver parenchyma. The primary lesion is, he thinks, a destruction of groups of liver cells at the periphery of the lobule. After such destruction the uninjured cells of the lobule, as well as the cells of the bile ducts proliferate in an attempt to repair the injury. The continuous occurrence of degeneration and regeneration causes the formation of new connective tissue which eventually atrophies, thus leading to the picture seen at autopsy. Cirrhosis, therefore, is the result of repeated localized destructive lesions from which the liver has more or less recovered. This view, it will readily be seen, accepts Weigert's [7] opinion that the primary lesion in all interstitial new growth of tissue is cell death, and also includes Kirikow's [8] opinion that in addition to cell death the continual action of the toxic substance is necessary; but adds the important factor of continuous regeneration.

W. G. MacCallum's [9] study of the regenerative changes in human cirrhosis strongly supports the theory of Kretz, as do also the studies by McPhedran and A. B. MacCallum,[10] Meder,[11] Marchand,[12] Stroebe,[13] Barbacci,[14] W. G. MacCallum,[15] and others of repair in acute yellow atrophy of the liver.

The experimental studies of repair after various mechanical injuries also indicate the ease and rapidity with which liver parenchyma repairs loss of substance. From the observations of Podwyssozki [16] and Ponfick [17] it is seen that slight injuries are repaired by the proliferation of either liver cells or the smaller bile ducts, connective-tissue repair occurring only when loss of substance exists. Of special interest is the true hyperplasia observed by Ponfick and by v. Meister [18] after extirpation of large portions (one-quarter to three-quarters) of the entire liver.

On the other hand, the argument is presented that focal necrotic lesions of the liver frequently occur in man without repair either by proliferation of parenchymatous cells or by formation of connective tissue. In this connection the hepatic lesions of typhoid fever, diphtheria, and other acute infections, as well as various experimental infections of animals, are quoted. It is probable, however, that in such cases the reparative power of the cells is in some way delayed, or consists possibly of a true regeneration of liver cells without the proliferation of connective tissue; for in hog cholera, in which, above all other diseases, necroses of the liver are most abundant and widespread, cirrhosis of the liver commonly occurs. Salmon, Smith, and Kilborne,[19] in the study of hog cholera in an epidemic including both acute and chronic cases, found well-marked cirrhosis in about one-half of the animals examined.

The question as to whether the interstitial tissue or the parenchyma is primarily affected in cirrhosis receives little enlightenment from the literature of experimental cirrhosis. Heukelom [20] who reviewed the literature up to 1896 collected nineteen different methods said to be capable of producing cirrhosis, and to these Joannovics [21] in a recent summary adds others. None, however, produces a lesion analogous to that occurring in man. These methods may readily be divided into two groups. In the first we have procedures essentially mechanical in nature, as ligation of the ducts or vessels of the liver, the injection of irritating substances into the ducts or directly into the liver substance, and their application to its surface. The second group includes the administration by the mouth, or by subcutaneous or intravenous injection, of various toxic substances. As a general rule, the mechanical methods lead to a more or less irregular connective-tissue formation, sometimes with considerable sclerosis, but the lesion under these circumstances is more of an atrophy than a cirrhosis. The toxic agents, for which positive results have been claimed, have been introduced so that they reach the liver through the blood stream. The lesion attributed to such substances is usually described as a primary degeneration of the liver cells with a slight increase of connective tissue limited, as a rule, to the portal spaces; in a few instances a definite perilobular growth of connective tissue, independent of parenchymatous degeneration, has been described. Few of these lesions, however, even in their final stages, are comparable to the cirrhosis of man, and in none has it been possible to follow step by step the development of a lesion which could properly be termed an experimental cirrhosis.

*Summary.*—The reparative process which follows the widespread necrosis of the dog's liver caused by the injection of hæmagglutinative serum constitutes a chronic interstitial hepatitis of definite and constant character. This is not only a new type of experimental hepatic lesion, but is more definitely a cirrhosis than is any other experimental lesion hitherto described. It is of importance in explaining the histogenesis of cirrhosis, and incidentally various repair processes in the liver; but it does not aid in the elucidation of the etiology of cirrhosis in man, nor does it explain the peculiar arrangement of the new connective tissue in any form of human cirrhosis except possibly that associated with chronic passive congestion. It definitely demonstrates, however, that cirrhosis may follow extensive primary destructive lesions, a view not yet fully accepted, and supports the contention of Kretz that cirrhosis is essentially a reparative process.

## EXPLANATION OF PLATE V.

Fig. 1.—The primary lesion. Extensive hyaline necrosis sparing only the tissues about the larger portal spaces. Thirty-eight hours.

Dose 1 : 1000. Methylene blue and eosin. No. 2 oc., No. 3 obj.—Leitz.

Fig. 2.—Experimental cirrhosis. Broad bands of newly-formed connective tissue surround the islands of normal liver about the portal spaces. In the stroma may be seen newly-formed bile ducts and numerous giant cells. Thirty-six days

Dose 1 : 1000. Hæmatoxylin and eosin. No. 3 oc., No. 3 obj.—Leitz.

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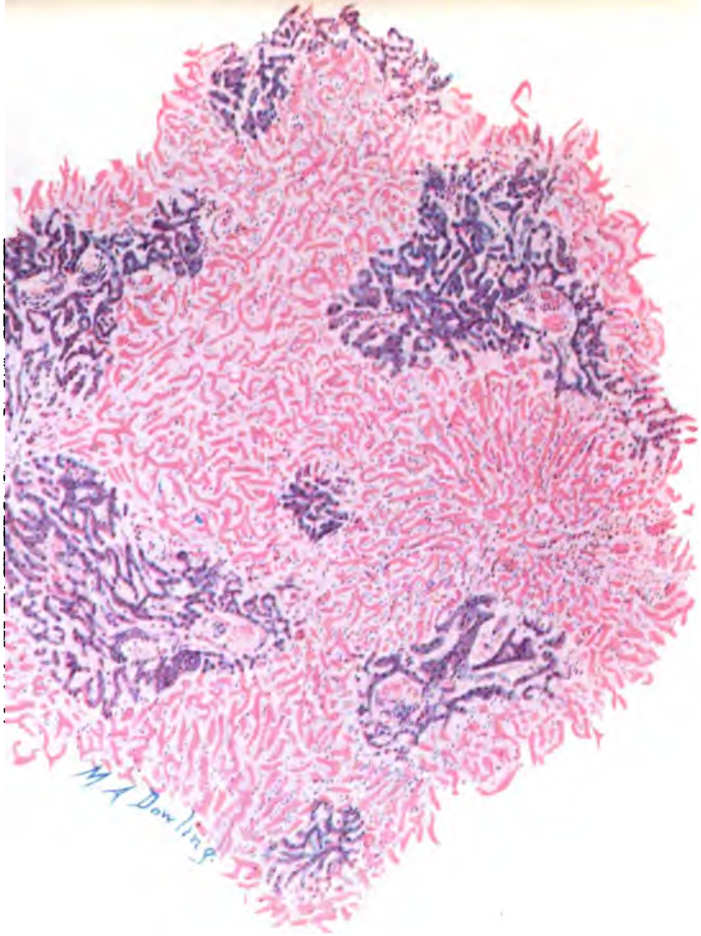

FIG. 1

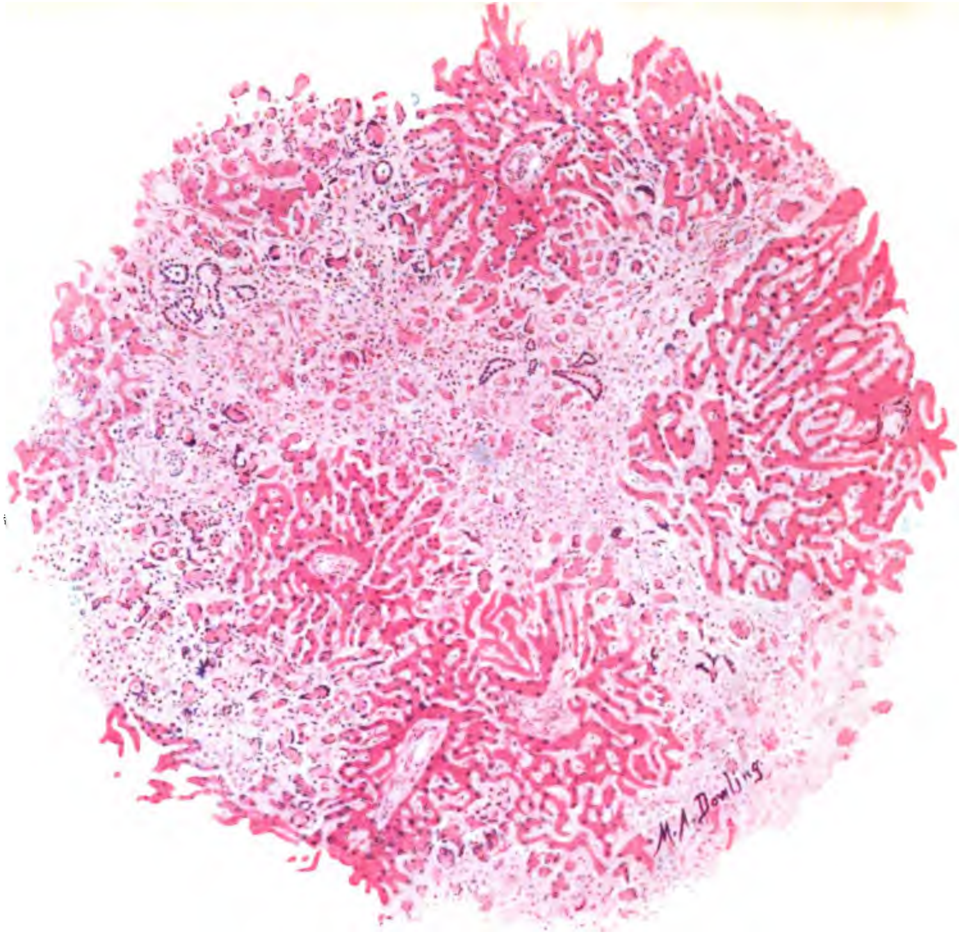

FIG. 2

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# EXPERIMENTAL ARTERIOSCLEROSIS.*

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Plates VI and VII.

The complexity of the pathological picture in the arteriosclerosis of man and the difficulty of determining the nature and sequence of the early changes have led many investigators to attempt the experimental production of the disease in the hope of thus solving some of its many confusing problems. Until within the last two years all such efforts have been unsuccessful. It is true, that Thoma described a diffuse arteriosclerosis in the dog as the result of a chronic experimental aortic insufficiency, and that Gilbert and Lion[1] produced scattered sclerotic and calcareous changes in the vessels of animals by injecting bacteria and their toxins. Thickening of the vascular walls has been described also as the result of poisoning animals with lead. These results, however, have been inconstant and lack confirmation. Jores,[2] in his excellent monograph on arteriosclerosis, published in 1903, reached the conclusion, based on a critical review of the literature, that all experimental methods fail to cause lesions similar to those occurring in man. Various endarterial lesions, obliterative or otherwise, as well as diffuse inflammation and atrophy, have been produced by injections of bacteria or their toxins, by the application of irritating substances to the perivascular tissues, and by ligation and other forms of mechanical injury; but none of these lesions is analogous to true arteriosclerosis.

It is a curious coincidence that in the same year in which Jores reached this conclusion, Josué[3] described experimental lesions

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in the aorta of rabbits, somewhat similar to those of human arteriosclerosis. These were caused by frequent intravenous injections of adrenalin. With the exception of a few inconclusive experiments of Jores, who gave dogs adrenal tablets in their food in the hope of obtaining lesions due to heightened blood-pressure, Josué appears to be the first investigator to attempt the experimental production of arteriosclerosis by the use of the principle of the adrenal gland. His results have been confirmed by Erb,[4] Rzentkowski,[5] Fisher,[6] and others. Their reports include but a small group of experiments and deal for the most part with the late changes, which they describe variously as arteriosclerosis, atheroma, and calcification. The principal histological changes to which they call attention are alteration in the elastica and the infiltration of lime salts.

The importance of a method capable of producing an experimental lesion so closely resembling human arteriosclerosis is apparent. It allows an opportunity to study many of the obscure problems of the disease, as the nature of the primary changes, the sequence of these changes, and the combinations which constitute the fully developed lesion. It was with the hope of elucidating some of these problems that the present investigation was undertaken. The results have been satisfactory beyond all expectation. Vascular changes capable of throwing much light upon the pathology of arteriosclerosis, and also that of aneurism, have been readily produced. Only a general outline of these will be given at this time, for many of the details of the histological changes, especially of the process of repair, are so important that they require a more extended study. A consideration of these details is therefore reserved for a future publication based on a second series of experiments now in progress.

*Methods.*—Rabbits have received in the veins of the ear repeated injections of a 1 to 1000 solution of adrenalin. The solution was prepared by a chemist with due regard to asepsis and chemical purity. Physiological salt solution was used as a medium. In addition to adrenalin (Parke, Davis, & Co.), it contained also chloretone in the proportion of one half of one

per cent., and a sufficient amount of hydrochloric acid to
the solution faintly acid.

A dose of three minims * repeated every other day ha
the usual procedure, though in some experiments the do
been gradually increased. Not infrequently the early inj
cause death from acute dilatation of the heart and puln
œdema. Such accidents usually occur after any one of th
seven or eight injections. If the animal survives this per
appears to gain a certain amount of immunity, or at least
ance to adrenalin, so that the dose may be gradually inc
during several weeks until from twenty to twenty-five n
may be given every day. This increased resistance to adr
has been noted also by Erb and Fisher. The animals hav
killed after periods varying from a few days to eight and
weeks.

For histological study, tissues have been preserved in Ze
fluid, alcohol, and six per cent. formalin, imbedded in cell
and stained with hæmatoxylin and eosin, and by Wei
elastica and Mallory's connective-tissue methods. The S
lach R. and osmic-acid methods have been employed fo
demonstration of fat. Sections hardened in Flemming's
tion and stained by hæmatoxylin have been found to be
satisfactory for purposes of general study. By this metho
nuclei and areas of calcification stain blue; fat, if present, l
the elastic fibres stand out prominently as light greenish-y
glistening lines, while all other structures are of a faint
color.

*Results.*—Of twenty animals receiving adrenalin, nine
cumbed to the acute effects of the drug within fifteen
These nine represent animals receiving one, three, four, five, s
and eight injections of adrenalin on alternate days. In
instances death resulted within a few minutes after the
tion; in others, after a few hours. The immediate effect

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* Throughout this report the dose will be given in minims. It wo
better perhaps to use the values of the metric system, but as Josué and
give the dose in minims or drops, we have for purposes of control us
same system of measurement.

intravenous administration of adrenalin is collapse with difficult and rapid respiration. The animal lies on its abdomen with legs outstretched and head resting on the table or raised in spasmodic respiratory effort; death, preceded by severe convulsive movements, may occur immediately. Other animals recover from the immediate manifestations only to succumb after a few hours. Upon post-mortem examination, the usual picture is acute dilatation of the heart and œdema of the lungs, with not infrequently small hæmorrhages beneath the pleura and pericardium; in one animal hæmorrhages were also found in the adrenal. These acute lesions indicate a very serious disturbance in the terminal vascular territories and are worthy perhaps of further investigation; but as our problem has to do only with the histological changes in the larger vessels its scope has been limited to that extent. The theoretical conclusions to be drawn from experimental adrenalin œdema and their possible application to acute œdema of the lung in man have recently been discussed by Josué.[7]

In but two of this group of nine animals were changes in the vessels demonstrable. These will be discussed later.

The second group of eleven animals represents those receiving from eight to twenty-eight injections during periods varying from sixteen to fifty-nine days. One of these died on the twenty-fifth day from spinal hæmorrhage; another on the forty-sixth day, from unknown cause, three weeks after the cessation of injections; the remaining ten were chloroformed. The aorta in six of these showed marked gross lesions; in the other five it was negative both macroscopically and microscopically. It is of interest that these five animals were all from the same litter and each weighed about 750 grammes at the time of the first injection. Pic and Bonnamour[8] have come to the conclusion that it is impossible to produce vascular lesions by adrenalin in rabbits weighing less than 2000 grammes, and quote in support of this opinion their negative experiments with animals weighing about 1200 grammes. That this is not an absolute rule is shown by the fact that the most characteristic and advanced lesions in our series occurred in an animal weighing 870 grammes at the beginning and 1020 at the end of the experiment. Othe however, our experience with small animals supports the tention of Pic and Bonnamour.

Attempts to produce lesions in dogs have been unsucce Both old and young dogs have been used. One of the latt puppy 2120 grammes in weight, received in the ear vein i month one hundred and thirty minims of adrenalin in rising gradually from four to twenty minims. Although res tory and cardiac disturbance frequently occurred immedi after injection, thus indicating a definite physiological a of the adrenalin, no evidence of vascular lesions could be f at autopsy or upon microscopic examination.

*Gross Lesions.*—These appear to be limited to the aorta involvement of other arteries as the brachial, carotid, and r which Erb describes, we have not found. The lesions in aorta are most marked in the thoracic portion and are sel found below the cœliac axis or in the first portion of the The earliest change in the vessel wall manifest to the naked is a faint longitudinal or irregular grayish streaking of the int without thickening. This appearance was seen as early as ninth day in a rabbit which had received five injections. A eight to fifteen injections, especially if the animal is allowe live for a week or longer after the last injection, very defi lesions are apparent. These consist of irregular, isolated confluent areas, usually slightly depressed, of a pearly color, and almost constantly calcified. The following prot illustrates this condition:

RABBIT NO. 11.—Weight, 2300 grammes. Killed May 2 after injectio follows: March 29 and 31, 4 minims; April 2 and 4, 5 minims; April 6 8, 7 minims; April 10, 10 minims; April 12, 12 minims; April 14, 15 16, 15 minims. *Autopsy.*—The aorta is distinctly dilated, measuring tw millimetres in circumference at the arch. Beginning at the origin of the carotid artery is an irregular patch, thirty-seven millimetres long, avera two and a half millimetres in width, and extending in a spiral course a the aorta for a distance of thirty millimetres so as completely to encircl Along this patch the wall of the aorta is slightly dilated. The media is parchment-like, and so distinctly calcified that the vessel cracks in sev places when the arch of the aorta is straightened. At one side of this patch are two very small oval areas of similar structure. There is no ather or ulceration. All the other organs are entirely normal.

The late lesions occurring after from twenty to twenty-five injections are very well marked. The aorta is more or less distorted, rigid, and non-elastic. Irregular dilatations alternate with elevated brittle areas of calcification. Distinct ulceration with atheroma is not readily demonstrable. Diffuse calcification is not infrequent and small aneurismal dilatations may be present. The following protocol illustrates the advanced lesions occurring in animals receiving large doses during a considerable period of time:

RABBIT No. 3.—Weight, 870 grammes. Received first injection of 3 minims in ear vein on November 30, 1904. Similar injections were repeated on alternate days until December 30, after which date the dose was rapidly increased until it reached 20 minims daily. The animal was killed on January 20, 1905. *Autopsy*.—Weight, 1150 grammes. The thoracic aorta measures six millimetres in diameter, is distinctly dilated, irregular in outline, and stiff and brittle, although the wall is apparently thinned. This condition extends as far as the cœliac axis, beyond which point the abdominal aorta is apparently normal. On opening the aorta the inner surface of the abdominal aorta above the cœliac axis shows numerous yellowish, slightly raised, calcified patches from one to one and a half millimetres in diameter, which in the upper part of the thoracic aorta become confluent. Except in one of the small patches situated in the upper portion of the abdominal aorta there is no evidence of ulceration. All other organs are normal.

The following description from the protocol of a parallel experiment illustrates the tendency to the formation of small aneurisms:

RABBIT No, 2.—The aorta averages three millimetres in diameter except at three places where distinct dilatations occur. The first of these begins three centimetres below the origin of the aorta and involves the right anterior wall of the aorta for a distance of two centimetres, giving the appearance of a fusiform aneurism; the diameter of the aorta at this point is four millimetres. A second dilatation, two and a half centimetres in length, occurs in the distal part of the thoracic aorta, and a third involves the abdominal aorta for a distance of one centimetre immediately below the cœliac axis. On section, the inner surface of the aorta shows sharply circumscribed saccular dilatations, varying from one to two millimetres in depth and corresponding to the protrusions described above. The walls of these aneurisms contain firm placques of calcareous material. The intervening aorta shows a number of yellowish-white elevated areas one to three millimetres in diameter. The aorta below the dilatation at the cœliac axis is macroscopically normal.

*Histology*.—In the group represented by the nine rabbits which died during the first two weeks, the vessels of but two showed histological changes. These were animals which received four and five injections respectively; the first dying the fifth day and the second on the ninth day. Scattered thro the middle coat of the aorta in each of these are small longitudi or occasionally irregular, finely granular foci of degeneration. these areas no nuclei are visible and the muscle fibres transformed into a finely granular, almost hyaline mate which stains deeply with eosin. The exact nature of this cha is not readily determined, but as the various methods of dem strating fatty transformation are negative, and those wh differentiate elastic fibres show no change in the staining react of the latter, the condition would appear to be a simple necro limited to the muscle fibres. The elastic tissue has, howe undergone very characteristic morphological changes which found only in the degenerated areas, and are due, presumab to mechanical influences. In the normal aorta the elastic fib have a distinctly wavy or curled appearance and are definit separated from one another; in the areas described the fib lose this appearance and become straightened and closely a proximated. They are also swollen and occasionally appear be fused together. Fracture of the fibres is seldom seen at t stage of the process.

A more diffuse lesion of this character was found in an anim killed on the twenty-fourth day after receiving eleven injectio The changes involve the entire circumference of the vesse sparing to a certain extent the innermost and outermost portio of the media. In this central zone few or no nuclei can be foun and the altered tissue presents a uniform, finely granular a pearance, relieved only by the glistening lines of the elas fibres. The latter by selective stain are seen to be so close massed together that individual fibres can be distinguished on with great difficulty. On either side of this zone the less d generated portions of the media show irregular areas which ta the hæmatoxylin stain in a manner very suggestive of early i filtration with lime salts. There is, however, no distinct calc fication and no fracture of elastic fibres.

Lesions of great interest in comparison with the above we observed in two rabbits which had received the same number of injections, but which were allowed to live for ten and eighteen days, respectively, after the last injection. In the aorta of each are found a few areas of granular necrosis, but for the most part these areas have been altered by infiltration with lime salts. From the topography of the areas of calcification and their relation to the necrotic foci, it is evident that the deposition of lime salts follows the necrosis. It would appear therefore that the primary degenerative lesions are well advanced by the end of the third week, and that after this but one or two weeks are sufficient for advanced calcification; the latter may, however, occur much earlier, for in one rabbit killed on the sixteenth day small but very definite foci of lime infiltration are present.

In the areas of lighter deposition of lime it is seen that these salts are deposited first between the elastic fibres, an observation which indicates that the destruction of the muscle fibre is the older, and therefore in all probability the primary, lesion. In the areas of advanced calcification, although the elastic fibres appear to be completely destroyed by the calcareous transformation, Weigert's stain shows them to be still present, though they stain poorly and are frequently fractured. Not uncommonly at a point of fracture one bundle of fibres overlaps another, the ends being splintered together by an encapsulating mass of lime salts. These fibres are always perfectly straight except at angles formed by fracture. A definite relation appears to exist between calcification and fracture of the elastic fibres. In areas of the most extensive calcification few breaks in the elastic fibres have been observed, while, on the other hand, they are constantly present in the aneursimal dilatations which show comparatively little calcification.

Lesions of this stage show the first evidence of repair. This is indicated by the collection of newly formed cells about the foci of calcification. These cells are closely massed, surrounded by but a slight ring of protoplasm, and appear to be of connective-tissue origin, though it has not always been possible to distinguish between such cells and the nuclei of smooth muscle fibres. No accumulation of polymorphonuclear leucocytes or lymphoid

cells has been seen. Of greater interest are the prolife
changes in the intima. These occur only opposite di
breaks or considerable depressions in the media, and in
proliferation of the lining endothelium and, to a greater e
of the subendothelial tissues. The endothelial cells proli
to form two or three layers of cells longitudinally arra
while the subendothelial space, barely visible in the n
aorta, becomes very prominent owing to the presence of c
packed oval nuclei arranged vertically to the lining endothe
Between the nuclei are delicate newly formed connective-
and elastic fibrils; in the late stages the newly formed e
fibrils are very prominent. In the angle of fracture the r
of the media assume a perpendicular arrangement very str
in contrast to their longitudinal arrangement elsewhere.
entire picture is distinctly that of a compensatory prol
tion: an effort to strengthen the weakened point in the
wall.

Such are the essential phases of this lesion in the ord
their sequence. The more prolonged experiments offe
fundamentally new features; but the combinations of
primary changes in the course of the more extensive inv
ment of the vessel wall lead to a complex histological pi
somewhat resembling advanced human arteriosclerosis. T
seen only in animals which have received gradually incre
doses during a period of seven or eight weeks. The vessel
becomes greatly thickened, not only by infiltration of lime
but also by an extensive repair process which involves the i
as well as the media. Small foci of complete necrosis, anal
to atheroma and entirely different from the primary dege
tion, are also seen in areas in which the elastic fibres are
pletely destroyed. Such foci present a uniform, finely gra
appearance and stain deeply with eosin; the osmic-acid m
for fat is negative, but a few fine droplets are evident after
ment with Scharlach R. About such areas repair takes
but it is not as active as it is about the masses of lime salts

The most striking feature of the late histological picture
extent of repair in the intima. The latter, with its newly fo

connective-tissue and elastic fibrils, becomes so greatly thickened that it constitutes in some places from a quarter to a third of the entire vessel wall, and offers convincing evidence of the compensatory nature of the repair process.

It is in these late lesions that the small aneurisms are seen. They occur at points where the elastica is so completely destroyed as to be transformed into an indistinct mass of fractured granular and fused fibres. The transition from normal to necrotic fibres is always sharp and distinct and marked by complete fracture of all elastic tissue. It is worthy of note that in the thinned wall of these aneurisms the degree of calcification is, as a rule, less marked than elsewhere; the adventitia is compressed, but otherwise unaffected.

The changes in other organs include enlargement of the heart, œdema and congestion of the lungs, and occasionally degenerative changes in the heart and skeletal muscles. In one case degenerative lesions were found in a nerve ganglion adherent to the aorta. It was thought that these changes might have some etiological relation to the vascular lesion, but a thorough study of all our material failed to reveal lesions in other animals, or in other ganglia of the same animal. The affected ganglion was in the adventitial tissue of an aneurismal dilatation, and the effects of pressure and disturbed blood-supply were sufficient, apparently, to account for the changes observed.

*The Mode of Action of Adrenalin.*—The manner in which adrenalin produces these lesions is a matter of widely varying opinion and, unfortunately, one which cannot readily be determined by our method of experimentation. Until we know more about the toxic action of adrenalin and can distinguish between the lesions due to this action and those due to increased blood-pressure, and especially between its direct and secondary effect on the blood-vessels, we can hope for no elucidation of this problem. Drummond,[9] who has recently made a thorough study of the histological lesions caused by adrenalin, divides them into those due to toxic action and those due to increased blood-pressure. It is obvious that such a classification is difficult, for it is impossible to determine to what extent degenerative lesions are due to interference with the circulation through ac the blood-vessels.

A discussion of the variety of ways in which adrenalin act, directly or indirectly, to cause degenerative changes media of the aorta would, in the present state of our kno be of little value. The difficulty of reaching definite conc is illustrated by the diversity of opinion expressed by tho have previously produced the lesions here described. believes them to be due to a combination of a specific to fluence and the increased blood-pressure; Rzentkowski b in the latter influence only; Erb ascribes an important in to disturbance of the *vasa vasorum;* Fisher thinks that from the increased blood-pressure, disturbances of meta play an important part; Lissauer [10] believes that the to fluence is the most important factor, but offers no expla of its mode of action.

No definite conclusion can be drawn from our own ments. The observation, that the earliest changes occur media and are apparently primary in the smooth muscle suggests, in view of the well-known physiologic action of alin on this tissue, a direct and selective toxic action; b absence of similar lesions in vessels other than the aorta d support this view. For the same reason, and also because absence of changes in the adventitia, the theory concerni *vasa vasorum* appears to be untenable. On the other h we assume, as is very probable, that the changes in for arrangement of the elastic fibres are due to the same fa factors which cause the destruction of the muscle cells, a not secondary to the latter, we have a strong argument i of the influence of a greatly heightened blood-pressure, f conceivable that these diffuse changes might result from tinued severe stretching of the vessel wall at the time ischæmia due to the primary action of the adrenalin. I words, it is possible that at the period of vascular spasm pr by the adrenalin the nutrition of the vessel wall is altered alone would not explain the limitation of the lesion to the but the added mechanical disturbance due to the extreme tion of the vessel wall would appear to be sufficient to bring about a condition analogous to anæmic necrosis. These problems, however, must be settled by other methods of experimentation. Although it is impossible to determine to what extent the primary changes are mechanical and to what extent toxic in nature, it is certain that some of the secondary changes, as the fracture of elastic fibres and the formation of aneurisms, are largely due to mechanical factors.

*Comparison of the Experimental Lesions and those Occurring in Man.*—If due allowance is made for the difference in size between the aorta of the rabbit and that of man, it must be admitted that the lesions produced in the former, as the result of the administration of adrenalin, are somewhat similar to those occurring in human arteriosclerosis. They are not, however, analogous. All the essential processes are represented and, considering the delicate character of the wall of the rabbit's aorta, the lesions are relatively as extensive and cause the same degree of deformity. There is, however, as compared with the human lesion, a difference in the initial change and in the degree of atheroma, which is insignificant and limited to the media. Extensive atheroma, however, cannot be expected in a vessel wall as thin as the rabbit's aorta, and, moreover, in none of these experiments has a period of time sufficient for the occurrence of degeneration in areas of excessive intimal proliferation elapsed. More prolonged experiments will yield, it is to be hoped, lesions more conclusive in this respect. For the present the condition may perhaps be regarded as arteriosclerosis of the rabbit, but not as a condition analogous to the arteriosclerosis of man.

In the light of the information gained from the study of these experimental lesions, it would be desirable perhaps to discuss critically the various theories concerning the nature of arteriosclerosis and especially the character and sequence of the histological changes. Such discussion, however, does not come within the scope of this communication, and many minor points must be determined before comparisons are justifiable. It is sufficient, for the present, to point out the strong support afforded Thoma's view, that the primary lesion of arteriosclerosis occurs

in the media and is, essentially, the result of changes in
tica; and that the alterations in the intima constitute
process the object of which is to compensate for the
media and the widened lumen.

## EXPLANATION OF PLATES VI AND VII

Fig. 1.—Gross appearance of a portion of the aorta from an an
had received twenty-seven injections and was killed on the twenty
The drawing is twice the actual size of the original specimen.

Fig. 2.—Diffuse degeneration of the central zone of media; eleven
twenty-fourth day. Hæmatoxylin and eosin; No. 4 oc., No. 3 obj

Fig. 3.—Microscopic picture of lesion pictured in Fig. 1. Destru
media with infiltration of lime salts; extensive proliferation of
Hæmatoxylin and eosin; No. 4 oc., No. 3 obj.—Leitz.

Fig. 4.—Elastic tissue stain of an area very similar to the above,
extreme destruction of the elastica.

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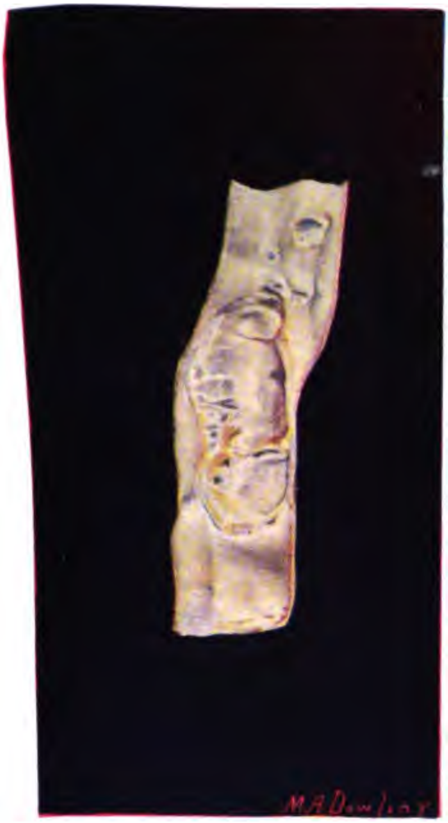

FIG. 1

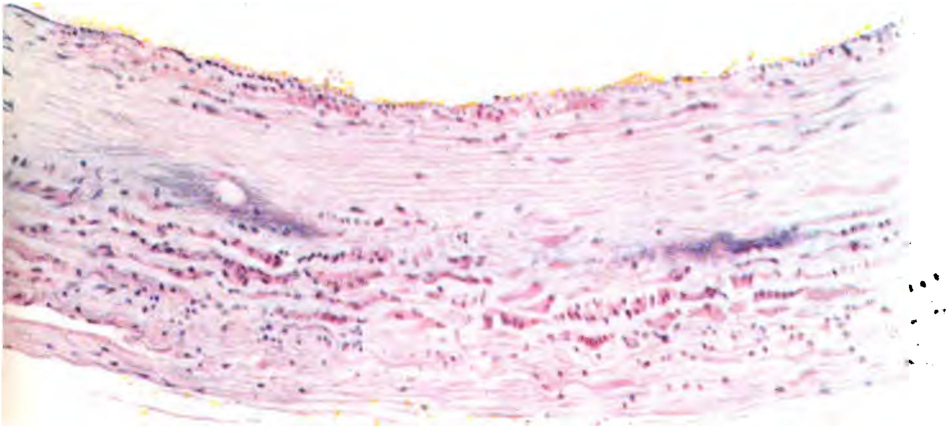

FIG. 2

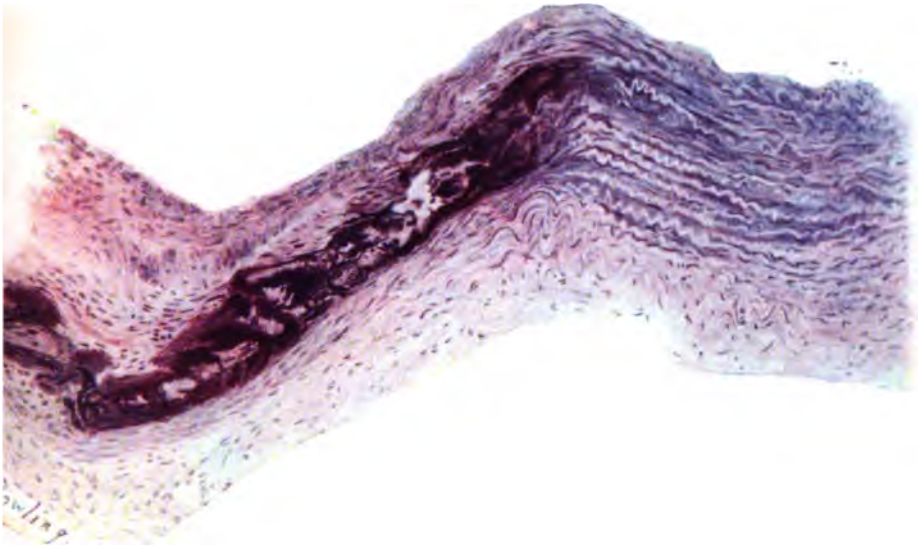

FIG. 3

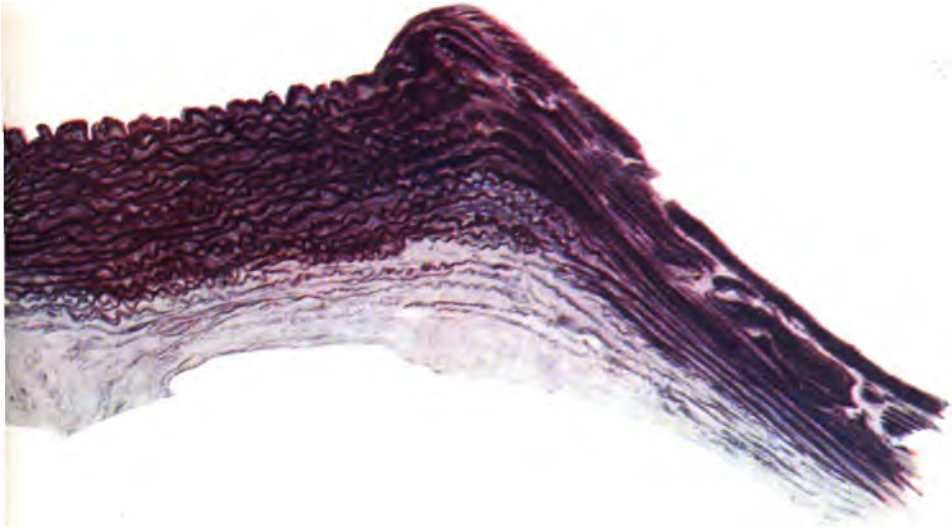

FIG. 4

# ON CERTAIN THERMOSTABILE VENOM ACTIVATORS.

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The nature of the cytolytic processes involved in venom cytolysis was made clear through the researches of Flexner and Noguchi, Calmette, and Kyes and Sachs. Flexner and Noguchi [1] first demonstrated that venom contains an intermediary body (amboceptor) for blood corpuscles and, later, they proved that venom also possesses intermediary bodies which are capable of uniting with the somatic cells of a wide series of animals and of bringing them under the solvent influence of extracellular and intracellular complements. The complements which they described are thermolabile bodies; while the researches of Calmette [2] pointed to the existence in serum of a thermostabile body which united with the venom hæmolytic principle and caused hæmolysis, and this body was discovered by Kyes and Sachs [3] to be lecithin. Recently Kyes has produced a crystalline substance which he calls cobra lecithid, which retains the property of bringing about solution of red blood corpuscles.

In order to study further the manner of action of lecithin in respect to its complementing property and to compare it with substances of related nature, Kyes tested other phosphorized fats such as kephalin, protagon, and cerebrin. He found that kephalin[4] was also capable of activating venom, although in less

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[1] Flexner and Noguchi, *Journal of Experimental Medicine*, 1902, vi, 277; *Journal of Pathology and Bacteriology*, 1902, viii, 379; *University of Pennsylvania Medical Bulletin*, 1903, xvi, 163.

[2] Calmette, *Compt. rend. Acad. d. Soc.*, Paris, 1902, cxxxiv, 1446.

[3] Kyes, *Berliner klin. Wochenschrift*, 1902, 886, 918; 1903, 956, 982. Kyes and Sachs, *op. cit.*, 1903, 21, 57, 82.

[4] I had also found that kephalin is complementary to venom, but I did not think it worth while to publish the observation after Kyes's paper appeared.

degree than lecithin, while the other two substances were
of this power. This fact is of theoretical interest, since k
stands closely related to lecithin chemically. On the othe
Kyes found that cholin, glycerino-phosphoric acid, and so
were inactive in this respect.

The nature of the case of venom activation calls for a
of the inherent hæmolytic powers of the substances emplo
activators, and this should be carried out in a strictly qu
tive manner. The great difficulty in the study of the hæ
power of lecithin is that this substance is very unstab
that it seems almost impossible to get a well-defined ch
body. Most of the commercial preparations of lecithi
prise a series of rather different bodies. The impression
that lecithin is an almost indifferent substance, while as a
of fact some preparations of lecithin are powerful hæm
agents. The source of misapprehension is to be found,
one hand, in the variation of the substance called lecithi
on the other, in the velocity of reaction, which is often overl
If we compare the hæmolysis caused by cobra venom pl
thin with that of lecithin[5] alone the final effect may be qu
same in both cases, but it is obtained much more quickly
first case. The difference may in some cases be so grea
the reaction is finished almost instantaneously with cob
lecithin, while it requires six hours or more for its com
with lecithin alone. I can point to other examples o
difference in the velocity of reaction, but not in the de
hæmolysis. Equivalent amounts of most acids produ
same final hæmolysis, but the rapidity is much greater
strongly dissociated acids.

Kyes studied the hæmolytic value of lecithin, and he
that, as compared with lecithin in the presence of venom,
200 to 300 times less active in the pure state. This esti
must be considered as conditional upon the manner of

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[5] Madsen and Noguchi, "Toxines et antitoxines," *L'influence de la ture sur la vitesse de réaction*, ii; *Académie royale des sciences et des l Danemark; Bulletin de l'année 1904*, 447; and *Centralbl. f. Bakt. und Pa* 1905, xxxvii, Abt. i., Referate, 356.

mentation. Venom-lecithin hæmolysis is complete within fifteen minutes, while simple lecithin hæmolysis is, as I have pointed out, complete only after about six hours. Hence, an absolute comparison of activity should be made not at once but after the lapse of at least this period. That it is essential to study the end reaction will be conceded when it is considered that the proportion at an early stage between lecithin hæmolysis and venom-lecithin hæmolysis may be as 1 to 1000, while at the end of the process it may be only as 1 to 20. A quantitative study of the reactions has indeed shown me that, as measured by the constant of the end of the reaction, the difference in hæmolytic activity of the pure lecithin as compared with the lecithin and cobra venom mixture is as 1 to 16. This proportion indicates the activating strength of the lecithin used by me; and a determination of the activating strength of other substances may equally be made by bearing in mind and observing the reaction velocity. It is in this way alone that any knowledge of absolute strength can be obtained. The present investigation had for its purpose the study of other substances than those already studied by Kyes with the view of ascertaining whether similar activating properties would be exhibited by any of them.

## METHODS.

*Materials.*—All the chemicals except lecithin were procured directly from Merck at Darmstadt immediately before being used. The lecithin was obtained, first, from hens' eggs, and was prepared by Dr. Madsen at this Institute; and, second, from the cardiac muscle of the ox, this sample having been prepared for me by Professor Bock at the Pharmacological Laboratory of the Copenhagen University.

The solutions were made normal, and employed as such with the few exceptions in which percentage solutions were used. All water-soluble chemicals were dissolved in distilled water, while the insoluble ones were emulsified in water by means of an equal weight of gum arabic. Lecithin, while insoluble in water, can be emulsified readily without the use of the suspending gum. Triolein, tripalmitin, and tristearin are not emulsified by these means; but, by first dissolving them in a small a of ether and then shaking them with a larger quantity of alcohol, they can be rendered emulsifiable. At least triol be suspended in this way, while tripalmitin and tristeari never rendered wholly satisfactory for experiment. The so of natrium oleinicum, natrium stearinicum, and natrium tinicum, on account of the liability to dissociation, were freshly prepared before being used.

The venoms employed were cobra, water moccasin, hab meresurus), and daboia. They were dissolved in 0.9 pe salt solution.

The blood corpuscles were those of the horse washed t salt solution and suspended in the proportion of 1 part condensed corpuscles to 99 parts of the 0.9 per cent. salt so These washed corpuscles are not hæmolyzed by any venoms alone. Hence, they are suitable for such an inv tion as the present.

*Test of Activation.*—Activation of venom can be accom by means of two kinds of substances: First, those whic no inherent capacity to cause hæmolysis, and, second, which, while causing independent hæmolysis, act in weak centrations in the presence of venom. In the latter ca velocity of the reaction is, as a rule, and especially with le accelerated. The thermolabile serum complements bel the first and lecithin and some other chemical substances second class of substances.

*Technique.*—Each tube contained 8 cubic centimeters 1 per cent suspension of condensed horse's corpuscles. T tube varying amounts of the substance to be tested for acti were added, and, at the same time, in the main series, 0. centimeters of a 0.3 per cent. solution of cobra or other The mixtures were first thoroughly shaken, then placed at for two hours, and kept fifteen hours longer at 15° C. Th ing was made at the end of this time, i. e., 17 hours. In suspended, insoluble substances, several shakings were during this period.

The estimation of the hæmolytic power was made on th of the extremes of action, namely, the quantity which sufficed to produce a trace and the quantity required just to produce complete hæmolysis. Control tubes, minus the activator and venom, were exposed in every series in duplicate exactly to the same conditions of shaking, temperature, etc.

The degree of dilution of the normal solutions was adjusted according to the activity of the substance. The stronger the native activity of the substance the lower the degree of normal strength, and *vice versa*. In this way the quantity of normal solution added was made to vary between 0.2 cubic centimeter and 1 cubic centimeter. In spite of their feeble power as activators, the higher fatty acids could not be used in stronger concentrations than $\frac{1}{10}$ N. on account of their high molecular weights; on the other hand, all the higher acrylic acids and their salts and lecithin[6] displayed such high power that they were employed in concentrations varying from $\frac{1}{100}$ N. to $\frac{1}{1000}$ N.

Table I gives the results with each substance tested,. the calculation having been made on the basis of 1 N. solution, unless special mention to the contrary is made. The sign — indicates no hæmolysis with the highest concentration employed.

An examination of this table will reveal certain new facts. Many acids produce hæmolysis, but a striking difference is apparent between the action of the normal fatty acids and the monocarbonic acrylic acids. The hæmolytic power of the normal fatty acids seems to become weaker with the higher members until, with some of the higher acids, no hæmolyzing effect is produced. The reverse is true of the acrylic monocarbonic acids, since with them the larger the number of carbon atoms the stronger is the hæmolytic power exerted by them.

The disappearance of the hæmolytic power in the higher

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[6] The molecular weight of lecithin is rather indefinite, but in this case I have taken it for a dioleinic lecithin ($C_{44}H_{86}O_9NP = 807$), and it was made into a 0.807 per cent. emulsion corresponding to $\frac{1}{100}$N. From this concentration any desirable quantity was made by means of addition of a certain quantity of 0.9 per cent. NaCl solution. The figures given in the tables are calculated on the basis of 1 N. in order to make possible the comparison of the results with those obtained with more weakly acting chemicals.

TABLE I.

| Name. | Native hæmolytic power. | | Hæmolytic power u venomized corpu | |
|---|---|---|---|---|
| | Dose for C.H. | Dose for Trace H. | Dose for C.H. | Do |
| Acid. formicum | 0.018 | 0.01 | 0.018 | 0.0 |
| " aceticum | 0.018 | 0.01 | 0.018 | 0.0 |
| " propionicum | 0.018 | 0.01 | 0.018 | 0.0 |
| " butyricum | 0.018 | 0.01 | 0.018 | 0.0 |
| " palmitinicum | 0.05 | 0.015 | 0.007 | 0.0 |
| " stearinicum | — | 0.08 | — | 0.0 |
| " arachinicum | — | — | — | — |
| " ceratinicum | — | — | — | — |
| Acid. crotonolicum | 0.025 | 0.015 | 0.005 | 0.0 |
| " nonylicum | 0.025 | 0.01 | 0.01 | 0.0 |
| " undecylenicum | 0.018 | 0.01 | 0.006 | 0.0 |
| " oleinicum | 0.002 | 0.0008 | 0.0002 | 0.0 |
| " elaidenicum | 0.02 | 0.0025 | 0.003 | 0.0 |
| Acid. ricinolicum | 0.015 | 0.002 | 0.003 | 0.0 |
| Acid. maleinicum | 0.013 | 0.008 | 0.013 | 0.0 |
| " fumaricum | 0.011 | 0.008 | 0.011 | 0.0 |
| " citraconicum | 0.015 | 0.008 | 0.015 | 0.0 |
| " itaconicum | 0.017 | 0.01 | 0.017 | 0.0 |
| Acid. sorbinicum | 0.017 | 0.01 | 0.017 | 0.0 |
| Acid. oxalicum | 0.014 | 0.006 | 0.014 | 0.0 |
| " succinicum | 0.012 | 0.005 | 0.012 | 0.0 |
| " tartaricum | 0.012 | 0.005 | 0.012 | 0.0 |
| " citricum | 0.008 | 0.0035 | 0.008 | 0.0 |
| Acid. glycerino-phosphoricum | 1. | 0.05 | 1. | 0.0 |
| Acid. hydrochloricum | 0.018 | 0.01 | 0.018 | 0.0 |
| " sulphuricum | 0.009 | 0.006 | 0.009 | 0.0 |
| " phosphoricum | 0.009 | 0.007 | 0.009 | 0.0 |
| Natrium stearinicum | 0.03 | 0.015 | 0.015 | 0.0 |
| " oleinicum | 0.0008 | 0.0005 | 0.0002 | 0.0 |
| " crotonolicum 1 % | 0.4 | 0.1 | 0.15 | 0.0 |
| Tripalmitin | — | — | — | — |
| Tristearin | — | — | — | — |
| Triolein | 0.0018 | 0.0006 | 0.00008 | 0.0 |
| Lecithin (egg) | 0.002 | 0.00065 | 0.00012 | 0.0 |
| " (ox's heart), unknown concentration | 2. | 1.2 | 0.2 | 0.0 |
| Neurin 1 % | 1.5 | 1. | 1.5 | 1. |
| Cholin 1 % | 1.5 | 1. | 1.5 | 1. |
| Alcohol methylenicus 10 N. | 2. | 1.5 | 2. | 1.5 |
| " ethylenicus 10 N. | 1.6 | 1. | 1.6 | 1. |
| " allylicus 10 N. | 0.5 | 0.3 | 0.07 | 0.0 |

normal acids may be due in part to their insolubility in water and in part to the reduction of the reaction velocity. That insolubility in water is not the only factor is shown by the behavior of the acrylic acids, which, while also insoluble in water, are, nevertheless, strongly hæmolytic. It is interesting to note that an isomer of oleinic acid, namely elaidenic acid, possesses much weaker action than the former acid, and, further, that ricinolic acid, the oxydate of oleinic acid, exhibits about the same power as elaidenic acid. It may be pointed out in this place that oleinic acid, sodium oleate, triolein, and lecithin possess the strongest hæmolytic action of all the chemicals studied, sodium oleate causing 2½ to 3 times the degree of hæmolysis produced by oleinic acid or lecithin, twice as much as triolein, and 22½ times as much as hydrochloric acid or the lower normal fatty acids.

With tripalmitin and tristearin no hæmolytic action was noted, although they were used in concentrated (1 N.) ethereal solutions, the volume of ethereal solution being, of course, limited to subhæmolytic amounts (to 8 c.c. less than 0.15 c.c.). However, the results with these substances are inconclusive.

The hæmolytic action of cholin and neurin is about the same, and the degree is about that of most monovalent acids. On the other hand, the power of the alcohols is weak, methyl alcohol standing below ethyl, and ethyl below allyl alcohol in hæmolytic power. It may be pointed out that the saturated alcohols tested were weak agents as compared with the far more active unsaturated allyl alcohol.

With this preliminary study of the hæmolytic activity of different chemicals we can proceed to a similar study of the same bodies in the presence of venom. Hitherto, there have been carefully studied, in this respect, only lecithin and kephalin and certain overheated serums in which the activating substances are probably of lecithin-like nature. Kyes, however, noted that a few neutral fats and certain salts of fatty acids activate venom in a minor degree.

Since I have shown that a relation or constant exists between the inherent and the venom activating hæmolytic power of certain chemicals, I felt the need of a standard with which any

of the active chemicals can be compared. For the purpo
this standard, I chose lecithin. According to my own ex
ments, lecithin possesses the following power: In
suspension the "complete" hæmolytic dose is 0.002; the "tr
hæmolytic dose, 0.00065. In the presence of cobra venon
"complete" dose is 0.00012, the "trace" dose, 0.00004. H
by the addition of cobra venom, lecithin is raised in powe
complete hæmolysis according to the equation $\frac{0.002}{0.00012}$, or
times, and for traces of hæmolysis, $\frac{0.00065}{0.00004}$, or 16.25 times.
since lecithin has been considered to be an activator in the
sense, as it combines with the venom hæmolytic ambocept
form a compound,[7] we may, for the present, call any subst
which is raised in power by venom in similar degree, a v
activator. Certain chemicals in the following table fulfill
requirement.

While the substances enumerated in Table II would ap
to be activating in an unmistakable way, they certainly
the requirements of my definition, although in different deg
The strongest body in this respect, of those tested, is tri
and as compared with the weakest body, nonylic acid, it is tw
times as powerful an activator. It is interesting to recall
the three strongest activators—triolein, oleinic acid, and
thin—are all related chemically to each other. That the
not act in a peculiar manner in the presence of venom an
not alone raised to multiples of their former hæmolytic str
through its presence, is shown by the conduct of such
activators as ricinolic, undecylenic, crotonolic, and perhaps
stearinic acid, which, while showing comparatively smal
herent activity, are raised in about the same proportion
tested in the presence of venom. On the other hand, the l
natrium oleate, which possesses the strongest inherent h
lytic powers, is one of the poorer activators. In the pre
of venom, oleinic acid is made as strong a hæmolyzing age
natrium oleate; in acting without venom the latter is two
half times as strong as oleinic acid.

The activity limits, for the production of traces and

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[7] *Cf.* Kyes, loc. cit.

TABLE II.

| | Complete hæmolysis. | | | | Trace of hæmolysis. | | | | Through venomization the hæmolytic power is increased by so many times. | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Without venom. | | With venom. | | Without venom. | | With venom. | | | |
| | Dose in c.c. 1 N. solution. | Hæmolytic units per 1 c.c. | Dose in c.c. 1 N. solution. | Hæmolytic units per 1 c.c. | Dose in c.c. 1 N. solution. | Hæmolytic units per 1 c.c. | Dose in c.c. 1 N. solution. | Hæmolytic units per 1 c.c. | For complete H. | For trace of H. |
| Lecithin.............. | 0.002 | 500.0 | 0.00012 | 8333.3 | 0.00065 | 1538.4 | 0.00004 | 25000.0 | 16.6 | 16.25 |
| Triolein.............. | 0 0018 | 555.5 | 0.00008 | 12500.0 | 0.0006 | 1666.6 | 0.000015 | 66666.6 | 22.5 | 40. |
| Acidum oleinicum..... | 0.002 | 500.0 | 0.0002 | 5000.0 | 0.0008 | 1250.0 | 0.00005 | 20000.0 | 10. | 16. |
| " ricinolicum... | 0.015 | 66.6 | 0.003 | 333.3 | 0.002 | 500.0 | 0.00015 | 6666.6 | 5. | 13.3 |
| " elaidenicum... | 0.02 | 50.0 | 0.003 | 333.3 | 0.0025 | 400.0 | 0.0002 | 5000 0 | 6.6 | 12.5 |
| " undecylenicum | 0.018 | 55.5 | 0.006 | 166.6 | 0.01 | 100.0 | 0.0004 | 2500.0 | 3. | 25. |
| " nonylicum.... | 0.025 | 40.0 | 0.01 | 100.0 | 0.01 | 100.0 | 0.005 | 200.0 | 2.5 | 2. |
| " crotonolicum . | 0.025 | 40.0 | 0.005 | 200.0 | 0.015 | 66.6 | 0.001 | 1000.0 | 5. | 15. |
| " palmitinicum. | 0.05 | 20.0 | 0.007 | 142.6 | 0.015 | 66.6 | 0.002 | 500.0 | 7.1 | 7.5 |
| " stearinicum... | 0.2 | 5.0 | 0.08 | 12.5 | 0.2 | 5.0 | 0.018 | 55.5 | 2.5 | 11 1 |
| Natrium " ... | 0.03 | 33.3 | 0.015 | 66.6 | 0.015 | 66.6 | 0.005 | 200.0 | 2. | 3. |
| " crotonolicum. | 0.04 | 25.00 | 0.015 | 66.6 | 0.01 | 100.0 | 0.002 | 500.0 | 2.6 | 5. |
| " oleinicum | 0.0008 | 1250.0 | 0.0002 | 5000.0 | 0.0005 | 2000.0 | 0 00005 | 20000.0 | 4. | 10. |
| Alcohol allylicus | 5.0 | 0.2 | 0.7 | 1.4 | 3.0. | 0.3 | 0.3 | 3.3 | 1.5 | 10. |

plete hæmolysis, are quite widely separated by venomizati increase being greater at the lower limits. The reason f difference has not been established, but it may to some be connected with the manner of distribution of the ins bodies in the fluid. In the presence of the smaller quanti insoluble substance a more minute distribution is possib tained, thus favoring union with the venom and action the corpuscles. What this difference depends on is at merely speculation; a special study would be required determination. The rate of hæmolysis in the presence of differs markedly for the different chemicals. With lecith reaction is rapidly produced, while with the rest of the acti it proceeds much more slowly. All the other hæmolyti stances tested, whether organic or inorganic, acted indiffe upon the normal and the venomized corpuscles. Possib ceptions are methyl and ethyl alcohols, in which a slight in of hæmolysis was observed in the presence of venom.

In the course of my work I have used other venoms cobra poison. I have not tested all the chemicals anew them, but have confined my studies to lecithin, triolein oleinic acid. The manner of carrying out the tests was sl modified. Twice-washed horse corpuscles (1 per cent per cent NaCl sol.) were mixed with uniform quantities three activators mentioned. The quantities employed we sufficient to produce inherent hæmolysis, but sufficient to about this change in the presence of venom, which was ad varying amounts. The mixtures were kept at 37° C. for two and at 15° C. for fifteen hours subsequently. The bloo pension was 8 cubic centimeters, the activator 0.0002 of solution, and the venom in 1 per cent solution of the poison, varying quantities per tube. Table III gives th tailed results.

It will be observed that all the venoms are activated three chemicals employed, and, excluding a few irregula these range themselves in the following order of activit olein, lecithin, oleinic acid. On the other hand, the re velocity is greatest with lecithin. One special fact may be

TABLE III.

| Kind of venom. | Cobra 1 %. | | Water Moccasin 1 %. | | Trimeresurus. 1 % | | Daboia 1 %. | | Crotalus 1 %. | |
|---|---|---|---|---|---|---|---|---|---|---|
| Kind of activator. | Dose for C. H. | Dose for trace H. | Dose for C. H. | Dose for trace H. | Dose for C. H. | Dose for trace H. | Dose for C. H. | Dose for trace H. | Dose for C. H. | Dose for trace H. |
| Lecithin 1 N. 0.0002. | 0.04 | 0.01 | 0.06 | 0.02 | 0.012 | 0.004 | 0.04 | 0.014 | 0.2 | 0.4 |
| Triolein 1 N. 0.0002 | 0.03 | 0.01 | 0.03 | 0.01 | 0.006 | 0.002 | 0.03 | 0.01 | 1.5 | 0.5 |
| Oleinic Acid 1 N. 0.0002 | 0.05 | 0.016 | 0.05 | 0.016 | 0.02 | 0.01 | 0.04 | 0.01 | 2.5 | 1. |

tioned about Crotalus venom. According to the results of these experiments, it appears to be a poor hæmolyzer, being 50 times weaker than cobra, water moccasin, and daboia venoms, and 100 to 150 times weaker than Trimeresurus venom. If, however, a more appropriate activator is employed, this difference disappears to a large extent. In the presence of dog's serum, whether fresh or after heating, the relative strength rises as compared with Trimeresurus as 1 to 10, and at the same time the reaction velocity is much accelerated. It is very probable that dog's serum, and perhaps other serums, contain thermostabile activators especially adapted to this venom. I might mention that several years ago I isolated from dog's serum, by ether extraction, a grayish hygroscopic powder, which was highly activating for Crotalus venom. I was unable at the time to identify this substance and I have not been able to return to its study.

As regards the quantitative relations of venom and activator, it may be said that the degree of hæmolysis depends on the relative quantities of each substance used. If to a given quantity of blood suspension more venom is added, then less activator is needed, and *vice versa.* Morgenroth and Sachs[8] studied the quantitative relations of serum hæmolysis, and Kyes has made observations on cobra venom and lecithin regarding the degree of hæmolysis, from which he concluded that the greater the quantity of venom employed the less the amount of lecithin needed. No study having been made of the more delicate quantitative relations of thermostabile activators and venom, I have tried, in this paper, to supply that deficiency. I have studied point of view cobra venom and three activators; namely, triolein, and oleinic acid.

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[8] Morgenroth and Sachs, *Ehrlich's Gesammelte Arbeiten zur Immunitätsforschung,* 1904, 359.

*Series A. Venom Uniform, Activator Variable.*—Each t tained 8 cubic centimeters of 1 per cent. horse's was puscles in 0.9 per cent. NaCl. The tubes, after recei venom and activators were kept at 37° C. for two h at 15° C. for four hours. Table IV gives the detail experiment.

TABLE IV.

| Cobra venom 1 %. | | 1 N. Lecithin. | | 1 N. Triolein. | | 1 N. Oleinic acid. | |
|---|---|---|---|---|---|---|---|
| Uniform dose for each set of tubes. | Proportion. | Amount for C. H. | Proportion. | Amount for C. H. | Proportion. | Amount for C. H. | Proportion. |
| i. 0.0025 | 1 | 0.0005 | 1 | 0.0003 | 1 | 0.0009 | 1 |
| ii. 0.005 | 2 | 0.00035 | 1:1.4 | 0.00025 | 1:1.2 | 0.0006 | 1:1.5 |
| iii. 0.01 | 4 | 0.00025 | 1:2.0 | 0.00016 | 1:1.9 | 0.0004 | 1:2.25 |
| iv. 0.02 | 8 | 0.00018 | 1:2.5 | 0.00012 | 1:2.5 | 0.0003 | 1:3.0 |
| v. 0.04 | 16 | 0.00012 | 1:4.1 | 0.00008 | 1:3.75 | 0.0002 | 1:4.25 |
| vi. 0.08 | 32 | 0.0001 | 1:5.0 | 0.00005 | 1:6.0 | 0.0001215 | 1:9.0 |
| vii. 0.16 | 64 | 0.000075 | 1:6.6 | 0.00004 | 1:7.5 | 0.0001 | 1:9.0 |

From this table the conclusion can be drawn that an in the quantity of venom does not reduce markedly the ment in activator, although it reduces the requirements atically. The definiteness of the relation between ven activator is striking in spite of a few irregularities, fo technical errors may easily account, and it may be expr the form of a rule which might be framed as follows: *quirement in activator for producing an equal degree of h of horse's corpuscles is in proportion to the square root of the of venom present in the mixture.*

*Series B. Activator Uniform, Venom Variable.*—This ment is the reverse of the preceding. All conditions same as in Series A, except that with a constant am activator, varying quantities of venom were employe results are given in Table V.

A study of this table suffices to show that the degree of the activator is different from that of the intermedia and that as the former is increased the latter may be di

### TABLE V.

1. LECITHIN + COBRA VENOM.

| 1 N. Lecithin. | | | Cobra venom 1 %. | | Square of proportion of the amount of lecithin. |
|---|---|---|---|---|---|
| | Uniform dose for each set. | Proportion. | Dose for C.H. | Proportion. | |
| i. | 0.00005 | 1 | 0.3 | 1 | 1 |
| ii. | 0.0001 | 2 | 0.08 | 1: 3.8 | 4 |
| iii. | 0.0002 | 4 | 0.02 | 1:15.0 | 16 |
| iv. | 0.0004 | 8 | 0.005 | 1:60.0 | 64 |
| v. | 0.0008 | 16 | 0.0015 | 1:200.0 | 256 |

2. TRIOLEIN + COBRA VENOM.

| 1 N. Triolein | | | Cobra Venom 1 %. | | Square of proportion of the amount of triolein. |
|---|---|---|---|---|---|
| | Uniform dose for each set. | Proportion. | Dose for C.H. | Proportion. | |
| i. | 0.00005 | 1 | 0.275 | 1 | 1 |
| ii. | 0.0001 | 2 | 0.08 | 1:3.4 | 4 |
| iii. | 0.0002 | 4 | 0.025 | 1:11.0 | 16 |
| iv. | 0.0004 | 8 | 0.0045 | 1:55.5 | 64 |
| v. | 0.0008 | 16 | 0.0012 | 1:230.0 | 256 |

3. OLEINIC ACID + COBRA VENOM.

| 1 N. Oleinic Acid. | | | Cobra Venom 1 %. | | Square of proportion of the amount of oleinic acid. |
|---|---|---|---|---|---|
| | Uniform dose for each set. | Proportion. | Dose for C.H. | Proportion. | |
| i. | 0.0001 | 1 | 0.225 | 1 | 1 |
| ii. | 0.0002 | 2 | 0.06 | 1:3.8 | 4 |
| iii. | 0.0004 | 4 | 0.0125 | 1.18.0 | 16 |
| iv. | 0.0008 | 8 | 0.003 | 1:75.0 | 64 |

without failure to cause hæmolysis. In this instance a rule may also be formulated, which might read: *An increase in activator permits of a reduction in venom approximately in proportion of the square of the amount of activator employed.*

*Inhibition of Lecithin-Venom Hæmolysis by Cholesteri* we[9] failed to observe definite protective action of c against venom-complement hæmolysis, Kyes[10] found lesterin is capable of preventing venom-lecithin hæm have experienced no difficulty in confirming this obser Kyes, and I have extended my study of the subject to tion as to whether cholesterin acts upon the venom ha or upon the activator.

The manner of study was as follows: A series of tube pared, containing venom in increasing and lecithin in d amounts. According to this plan and in keeping with servations made in an earlier part of this paper, several hæmolytic mixtures will be obtained. To these mixtur terin is added in exactly the quantity needed to prevent sis. If the amount of cholesterin required is propor the venom used, the presumption would naturally b reacted with that substance, while if it is proportion lecithin, then the presumption would be in favor of with that body. Should the amount of cholesterin rema constant throughout the experiments, the probability that it acted upon the blood corpuscles and protect directly from hæmolysis.

Five series of tests were made. The orders were as i. Venom + lecithin + cholesterin + blood; ii. venom + ch + lecithin + blood; iii. lecithin + cholesterin + venom + b blood + lecithin + cholesterin + venom; v. blood + venom terin + lecithin. The results of the tests are given in which follows.

Table VI shows that a relation exists between the ch and lecithin in the mixtures and not between cholest venom or corpuscles, and this fact seems to prove con that the antihæmolytic action of cholesterin depends o action with lecithin. In order that lecithin-venom h may be prevented by cholesterin, a certain interval between the lecithin and cholesterin is required with

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[9] Flexner and Noguchi, loc. cit.

[10] Kyes, loc. cit.

TABLE VI.

| | Combination of venom, lecithin, cholesterin,* and blood suspension. | Cobra venom 0.2 % plus lecithin 1 N. | Correlative amounts of venom and lecithin, representing units of hæmolytic power. | | | |
|---|---|---|---|---|---|---|
| | | | 0.1<br>0.0004 | 0.2<br>0.0003 | 0.4<br>0.0002 | 1.6<br>0.0001 |
| i. | Venom + lecithin, 6 hours—36° C. + *cholesterin*, 15 minutes—15° C. + blood. | Takes 1 N. cholesterin. | 0.0008 | 0.0006 | 0.0004 | 0.00016 |
| | " + " " " + " 3 " —15° C. + " | " " " | 0.0012 | 0.0010 | 0.0007 | 0.00036 |
| | " + " 3 min. —36° C. + " " " " + " | " " " | 0.0012 | 0.0010 | 0.0008 | 0.0004 |
| ii. | Venom + *cholesterin*, 6 hours—36° C. + lecithin, 15 minutes—15° C. + blood. | " " " | 0.0008 | 0.0006 | 0.0004 | 0.00016 |
| | " + " " " + " 5 " " + " | " " " | 0.0012 | 0.0010 | 0.0008 | 0.00032 |
| | " + " 3 min.—36° C. + " " " " + " | " " " | 0.0012 | 0.0010 | 0.0007 | 0.00036 |
| iii. | Lecithin + *cholesterin*, 6 hours—36° C. + venom, 15 minutes—15° C. + blood. | " " " | 0.00076 | 0.00058 | 0.00036 | 0.00016 |
| | " + " " " + " 3 " " + " | " " " | 0.0008 | 0.0006 | 0.00036 | 0.00016 |
| | " + " 3 min. —36° C. + " " " " + " | " " " | 0.0012 | 0.0010 | 0.0006 | 0.00036 |
| iv. | Blood + lecithin, 60 min.—36° C. + *cholesterin*, 15 minutes—15° C. + venom. | " " " | 0.0008 | 0.0006 | 0.0004 | 0.0002 |
| v. | Blood + venom, 30 min.—36° C. + *cholesterin*, 15 minutes—15° C. + lecithin. | " " " | 0.0018 | 0.0012 | 0.0008 | 0.0004 |

* The amount of cholesterin used was contained in 0.2 c.c. of methyl alcohol.

quantities of cholesterin, while with larger amounts of thi stance the time interval is greatly diminished.

It is probable that the reaction between cholesterin and le depends on their solubilities in each other, and, hence, conditions are so modified as to alter essentially their solub the result may be quite or wholly changed. That this i can be shown by the action of methyl alcohol, which is ca of setting aside the antihæmolytic action of cholesterin. if 0.4 cubic centimeter of methyl alcohol is added to 8 centimeters of the mixture of horses' corpuscles, venom lecithin, the quantity of cholesterin required to prevent hæ sis will be greatly increased, while a quantity of the a over 0.8 cubic centimeter so changes the conditions as to r cholesterin impotent in preventing, although it may still hæmolysis as Table VII exhibits.

TABLE VII.

| Hæmolytic mixture. Amount of methyl alcohol. | Cobra venom 0.2 % Lecithin 1 N. | 0.1 0.0004. | 0.2. 0.0003. | 0.4. 0.0002. |
|---|---|---|---|---|
| 0.1 | Cholesterin 1 N. | 0.0007 | 0.00056 | 0.0004 |
| 0.2 | " " | 0.0008 | 0.0006 | 0.0004 |
| 0.3 | " " | 0.0010 | 0.0008 | 0.0005 |
| 0.4 | " " | 0.0025 | 0.0020 | 0.0015 |
| 0.5 | " " | 0.0060 | 0.0040 | 0.0028 |
| 0.6 | " " | 0.0100 | 0.0080 | 0.0050 |
| 0.7 | " " | 0.0400 | 0.0300 | 0.0200 |
| 0.8 | " " | Hæmolysis retarded, but no prote | | |
| 1.0 | " " | " | " | " " |

This study was carried out while I was being maint abroad by a grant from the Carnegie Institution of Washir D.C., and it is a continuation of the studies on venom wh began at the University of Pennsylvania with Professor Fle in which I had the support and advice of Dr. S. Weir Mitch

I desire to thank Dr. Madsen, the director of the St Serum Institute, for many acts of kindness during my stay laboratory.

# A FURTHER CONTRIBUTION TO THE KNOWLEDGE OF MULTIPLE NON-INFLAMMATORY NECROSIS OF THE LIVER WITH JAUNDICE (HEPAR NECROTICUM CUM ICTERO), AND TO THE KNOWLEDGE OF CELL DEGENERATION AND CYTOLYSIS IN GENERAL.[1]

BY HORST OERTEL.

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PLATE VIII.

In a previous communication [2] I described under this title the anatomical and histological findings of a peculiar affection of the liver, which could not be grouped under any of the headings now in general use in the classification of the diseases of that organ, and which presented interesting general pathological features in the form of a peculiar cellular destruction, unassociated with any inflammatory reaction and unlike that usually observed in the disintegration of parenchymatous cells.

To recapitulate, the process consisted of a multiple, irregular, circumscribed solution of liver cells, without parenchymatous degeneration or coagulation necrosis, and associated with a corresponding lobular blood and bile stasis in the affected areas. It appeared that the protoplasm and later the nucleus of the cells simply wasted and dissolved, leaving a more or less well-preserved reticulum with stagnant bile and blood. While undergoing this change the cells showed fatty degeneration, and finer or coarser bile pigment within their bodies, sometimes reaching a considerable degree, conditions to which I shall refer in detail later. The impression one gained from the appearance was that of a solution or cytolysis of the parenchyma. The process was as a rule more marked in the central portions of the lobules, taking its origin around the central vein, but by no means always confined to that point, as it occurred also in streaks running from one into neighboring lobules, and gradually was lost in well-preserved liver tissue. Moderate peripheral fatty infiltration existed, but there was an entire absence of inflammatory reaction within the liver tissue

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[1] This study was aided by an appropriation from the Rockefeller Institute for Medical Research.

[2] *Journal of Medical Research*, 1904, xii, 75.

itself, the portal spaces alone showing sclerosis of a more or less rece
The last condition was most pronounced and mature around the po
itself.

The whole lesion presented itself as a diffuse affection of the liver,
not led to any marked gross diminution in size, but the organ had
tough, leathery, pale yellow, bile stained, and the markings had b
Irregular vascular injection or darker, larger, deep red spots (correspo
the irregular stasis) were plainly visible.

Two features then made this observation of particu
terest. First, the peculiar destruction of liver cells wh
not correspond to the usual atrophy, degeneration, or r
of parenchymatous cells, second, the entire lack of ac
chronic inflammatory reaction of the liver tissue, for the a
areas showed only the passive feature of the cellular
Further, the well-confined sclerosis of the portal spaces wa
mature around the vessels than around the proliferati
ducts.

I explained in my first communication that we had here a condit
could not be grouped within the present classification of the disease
liver, and on account of the general severe destruction of liver tissue
generalized jaundice I suggested the term of multiple non-inflamma
crosis of the liver with jaundice (Hepar necroticum cum ictero).

In looking through the literature for similar or related cases I was a
to find a number of observations by Curschmann,[3] under the title of a
form of necrotic hepatitis, under which name is described, as followi
continued obstructive jaundice, somewhat similar multiple non-inflar
destruction of liver tissue, but leading to actual softening and breaki
of circumscribed areas. Here also the peculiar feature of a simple loss
substance with bile imbibition and an entire lack of inflammatory rea
or around the affected areas attracted his attention, as well as a well
scribed but confined sclerosis of the bile ducts of the portal spaces.

Curschmann, however, could not give any evidence as to how the cel
struction took place in his cases, for they were all far advanced be
stage of studying the histogenesis of the lesion. But in the explanati
cases Curschmann expresses an idea which I utilized in the explanatio
case. It was based largely on the clinical history, but also on some of t
tional anatomical and histological findings. He believes that his case
be understood on the basis of bile stagnation alone, but that it is nec
assume an additional infection, and recalls that certain bacteria, like
terium coli introduced into the bile channels, do not necessarily prod
puration, but a simple general infection. In the case that I had obs

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[3] "Ueber eine eigenartige Form der nekrotisirenden Hepatitis,"
*Archiv. für klinischen Medizin*, 1899, lxiv, 564.

had to do with an individual addicted to chronic alcoholism who, although a young man, showed mental and anatomical alterations incident to such abuse; and I assumed that in such cases a portal infection acting in a peculiar manner might easily occur and reach the liver, which as a consequence of constant exposure to harm was of low resistance and reacting ability. In favor of this portal entrance of an irritant was the marked localized sclerosis around the portal vessels, analogous to the thickening of the bile ducts in Curschmann's cases.

I advanced at that time no positive opinion as to the exact pathogenesis of the lesion, but it was natural to suppose that it was of bacterial origin, and the peculiar cytolytic changes as well as the entire absence of inflammatory reaction were somewhat more in favor of the action of a bacterial product than of a bacterium itself. However, in the absence of more data I did not enter into detailed discussion of the facts, the apparent rarity and peculiarity of the process inducing me to place it on record as an individual case.

Since that time, that is, for a period of a year and a half, I had particular attention paid in this institute to the various affections of the liver, and we have been able to collect three more cases that belong to the same category, which I now wish to add to my first contribution to the subject, as enlarging upon this peculiar and little appreciated affection. For reference and sake of convenience I may be allowed to quote and somewhat enlarge upon the findings of the first published case.[4]

*Case I.*—M. G., Irish, longshoreman, thirty years old, was admitted to the City Hospital, Oct. 8, 1903. He had been drinking heavily since the beginning of July, and was in an extremely nervous and mentally somewhat confused condition. His appetite was good, but he vomited some, had night sweats, and a slight cough. His previous history was that he had always been a very heavy drinker, and that he had been confined in bed on several occasions because of alcoholism. The only other disease which he admits is bronchitis. Both parents and one brother died of tuberculosis.

*Physical Examination.*—Patient jaundiced, but of good nutrition and development. Lungs reveal nothing of importance. Heart: enlarged to percussion from right sternal line to a point slightly beyond the apex beat; systolic murmur at apex. Liver and spleen not felt. Extremities; pretibial pitting. Temperature, 98°. Pulse, 100. Respiration, 24. Urine, reddish yellow, small amount, 1.025, no albumin, no sugar, no casts. During the next two days there was no material change. Temperature varied from 99° to 98°. Pulse, 100 to 104. Respiration, 24. Mind not improved. Jaundice somewhat more marked.

On the third day the patient became violently delirious, and was difficult to restrain, behaving like a case of delirium tremens. His temperature w 100°; and on the fourth day to 104°. Pulse, 120. Respiration, 32. of marked œdema of the lungs, on October 13, 5 A.M., having been in pital not quite five days.

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[4] In these histories details apparently not bearing on the question under consideration have been omitted.

*Autopsy Protocol* (abbreviated).
(Oertel) Oct. 13th, 2 P.M.

Male, thirty years old, one meter seventy-eight centimeters tall, of gc and muscular development, and good nutrition. Skin of the whole l conjunctivæ distinctly jaundiced. No œdema. Rigor mortis marke erate amount of subcutaneous fat, muscle tissue dry and brown. P cavity empty. Pericardium almost empty. Right heart collapsed, v fluid blood, left heart moderately contracted and empty. Pulmonary cuspid valves intact, aortic and mitral valves thickened at base. He cle brownish red, moderately firm. Weight, 555 grams. Left pleur without fluid or adhesions. Left lung moderately emphysematous, section, deep red, and very œdematous. Right pleura with dense, adhesions throughout. Lung markedly emphysematous and œdemato

*Spleen.*—Weight, 195 grams. Capsule thickened, firm. Section c connective tissue prominent, lymphoid nodules indistinct. *Kidneys* Slightly enlarged, firm, capsule thickened, moderately but uniformly a surface mottled, markings distinct. Cortex irregular. (Right).—San *renals* unchanged. *Stomach.*—Large, dilated with gas, but empty, ex much slimy material. Mucous membrane thickened, much congested *tines.*—Like stomach. Portal structures free.

*Liver.*—Somewhat larger than normal, weight, 1770 grams; color, dar throughout. Surface on general view smooth, but on closer inspection irregular; consistency firm, leathery. On section cuts firm, vessels dist deep red in many places, in others not prominent. Lobules pale yello spots brownish, their outlines indistinct and irregular. In some places presents a diffuse, yellowish pale appearance, but always firm, nowl softening. No connective-tissue bands visible. The gall bladder co fair amount of thick, fluid bile; no stones; the larger bile ducts perme dilated, and with no apparent change. The vessels at entrance into liv sively thickened.

Other organs without important alterations.

Microscopic examination of the liver reveals a most u picture. The stained sections show even to the naked eye irregular streaks of pale, poorly stained areas. On exami these areas (Fig. 1) are found to correspond to wasted p of liver lobules, usually with a free precipitation of bile form of large and small light yellow to dark brown gr The process leading to loss of the cells is a simple one, w coagulation or extensive parenchymatous or fatty degener being evidently more like a solution of the protoplasm, preceded by moderate parenchymatous swelling. In my first report I spoke of it as an atrophy. But it differs from ordinary atrophy in a much more rapid, uniform loss of the cellular protoplasm, while the outline of the cell remains visible to the last. One gains the impression that in the affected areas the cellular protoplasm is washed away. The nucleus becomes poorer in chromatin and atrophic, the cells become vacuolated, some contain fat drops, and the cell body then usually encloses smaller or larger granules of bile. Ultimately the cell disappears and only a fine reticulum remains with free bile pigment. The condition is most pronounced around the central vein, which appears well dilated. Towards the periphery of the lobule the cells have retained their integrity, but most of them show beginning turbidity and swelling of the protoplasm, with moderate fatty infiltration. They are also frequently seen to contain fine or coarse granular bile pigment. Their nucleus is poor in chromatin. Such areas are present in immense numbers in various portions of the liver, varying in size and shape, and are directly continuous with apparently normal liver tissue. They are not surrounded by an area of pus or any other infiltration. There is no line of demarcation anywhere, no inflammatory engorgement of vessels, no connective-tissue growth, old or recent, between or within the lobules—in fact, no evidence of any reaction of the liver tissue at all. There is present, however, a pronounced stasis in many places. But this bears no relation to the degree of atrophy or the precipitation of bile, as one may be more pronounced when the other is less conspicuous. In some places, where the waste of the whole central part of the lobule is pronounced, the stagnant blood occupies the space, either free or mixed with bile pigment. The stasis is irregular, not general, not uniformly distributed throughout the whole liver, corresponding, then, to the gross appearance.

Attention is attracted by a characteristic change of the portal spaces, confined to them. The portal vein is generally widely dilated, its walls well thickened, and its neighborhood densely infiltrated with small, round, and connective-tissue cells.

The same is true of the bile ducts and the hepatic vesse connective-tissue formation around these structures is fre more recent and more cellular, than immediately aro portal vein. The sclerosis is confined entirely to the st of the portal spaces, at no time is there an attempt to surr invade the lobules. It appears independent of the lesion lobules. In some places proliferation of bile ducts, whi reach a considerable degree, is going on. The new d well formed, their epithelium intact, and not congest bile.

*Case II.*—D. B., Irish, shoemaker, 48 years old, was admitted to Hospital, October 7, 1904, complaining of shortness of breath. He fir this disturbance about four weeks ago, when he coughed up loose phle his cough became tighter, and he could hardly expectorate at all. At intervals he also suffered from stabbing pains in the chest, preventing drawing his breath. His family history is negative. The patient ha ordinary diseases of childhood; eighteen years ago suffered from as bronchitis and since that time has had similar attacks every fall an He admits gonorrhœa many years ago, but denies syphilis. Had be whisky and beer drinker, and smoker, all his life.

*Physical Examination.*—Patient lies in bed quite dyspnœic, with hands deeply cyanosed and a slight unproductive cough. At inter mildly delirious. Chest shows signs of wide-spread emphysema, parti the bases. Heart dullness almost entirely obscured by hyper-reson the sounds distant. Rhythm irregular, the second sound at the ape cated. Liver enlarged downward to level of umbilicus; tender. Sp palpated, and knee jerks cannot be elicited.

Urine: amber, cloudy, acid. Sp. gr., 1.030. Trace of albumin, no casts. Temperature, 99° F. Pulse, 128. Respiration, 54. On the the patient was bled, about 300 c.c.blood being removed, and his chest his bowels were regulated, and strychnine administered. Under this t he improved somewhat, but soon relapsed, his temperature rose to 102°, the delirium became more marked, and he died five days after a October 12, 1.30 P.M.

*Autopsy Protocol* (abbreviated).
(Symmers) Oct. 12th, 3 P.M.

Body about 172 cm. long, good frame and muscular development nutrition. Skin pale and in parts slightly yellowish, conjunctivæ w yellow tinge, no external œdema. On section subcutaneous fat well p muscle tissue dry and pale brown. Abdominal cavity without flui intestines in lower part of belly, collapsed, smooth. Liver visible i line and right hypochondrium where it extends 5 cm. below the free the ribs, 10 cm. below the sternum in the median line. Diaphragm 4th interspace on right, and 5th on the left side. 100 c.c. fluid in the right pleural cavity, left pleural cavity obliterated. Pericardium contains 25 c.c. of fluid, but free.

*Brain.*—General marked atrophy of gray matter and thrombotic endarteritis. *Heart.*—With marked dilatation and hypertrophy of the right ventricle, muscle pale and cloudy. *Lungs.*—Marked general emphysema, chronic bronchitis, and irregular œdema. *Spleen.*—Small, atrophic, pulp very dark and prominent, *Kidneys.*—Large, juicy, dark. Cortex with distinct dark glomeruli; swollen, medulla œdematous. *Stomach.*—With well preserved rugæ, and thick, swollen mucous membrane, covered with mucus, and reddened near pylorus. *Pancreas.*—Large, narrow, firm, and pale. On section, pinkish.

*Liver.* —Somewhat smaller than normal and with localized thickening of capsule. Mottled, partly reddish-blue and partly yellowish-pink surface, but smooth, firm, and leathery. On section it shows towards the periphery of the organ a margin of spotted to more streaky dark vascular injection, but no well defined markings. Towards the centre and including the greater part all markings are lost, and the tissue appears of a diffuse pale yellow color with pointlike, numerous deeper yellow dots, irregularly distributed throughout the whole of the liver substance. The organ is of peculiar firm leathery consistency, but shows nowhere any softening.

Microscopic sections of the liver show to the naked eye an irregular streaky appearance due to an interchange of better and poor stained, pale areas. The pale portions present pinhead to more elongated shapes, and appear well circumscribed. On microscopic examination they correspond to lost or disintegrating groups of liver cells of a peculiar nature (Fig. 2): The protoplasm of the cell loses its staining quality, assumes a hazy, distant appearance, and usually presents moderate irregular swelling; nucleus and protoplasm become then quite indistinct, fade away, larger and smaller fat drops may appear within the protoplasm, the cellular substance seems to dissolve and disappear, but during the whole process the cellular outline is preserved and distinct to the last. Where no fatty change appears the nucleus remains central, where large fat drops occur it is pushed to one side. Ultimately only "ghosts" of cells remain, or a faint, slight reticulum, or even open free spaces which may contain fat, blood, and bile arise. However, blood and bile stasis is here much less pronounced and prominent than in Case I, the wasting cells and subsequent necrotic portions showing a more moderate bile imbibition.

The distribution of the lesion is general, diffuse, but irr
throughout the whole of the liver. Although frequently
its origin around the central vein where it appears promi
is not so well confined to the central portions of the lobul
the first case. It appears more irregular and streaky,
same gradual change to well preserved liver tissue exists
disintegrated areas show few free small spindle and
nuclei, some leucocytes and nuclear remnants. Nowhere i
evidence of any inflammatory reaction. The portal spa
here only moderately thickened, the vessels large, an
sclerosis is more marked around the bile ducts. A limited
bile duct formation coexists. The uninvolved parts of th
tissue show in part a moderate simple atrophy, but wit
preserved and well stained cells, in part slight cloudy sw
and some fine round and more elongated nuclei may be ob
within the lobules. Similar changes are not found in
organs, nor are features present elsewhere that could be b
into connection with the peculiar destruction of the liver.

*Case III.*—M. E., colored, 28 years old, was admitted to the City
as a transfer case from Harlem Hospital on October 8, 1904. She h
operated upon at that Hospital for what was diagnosed as cirrhosis of t
a ventrofixation of the omentum having been performed. Patient
up to the latter part of August, when she began to feel ill, with loss of
and flesh. She states that she had several hæmorrhages from the lu
was taken care of in another hospital and improved. Soon after goin
her abdomen began to swell, she became very short of breath, and
frequently. On going to Harlem Hospital she was operated upon, but
regain strength, and was therefore transferred to the City Hospital. S
plains now of weakness and very poor appetite. Her previous histor
that she has always performed hard domestic work, and that her childh
been healthy, but she never was very strong; the menses started at
and were always regular. She married at 18, has two children, and
miscarriages about the seventh month. Family history negative.
holic or syphilitic history.

*Physical Examination.*—General nutrition poor, skin dry and scal
moderate pretibial œdema. Cheeks puffy, lips cyanotic, and external
veins greatly engorged. Heart enlarged, extends to outside the nipple
to right of sternum. A loud blowing systolic murmur at the apex tran
to the axilla, aortic second sound accentuated. Pulse fast and irregular.
emphysematous. Liver felt below costal margin, spleen not palpable.
light yellow, sp. gr., 1.008. Albumin, trace; few hyaline casts. The
became rapidly worse, her œdema became more pronounced and gene

the abdomen began to fill; breathing was more and more labored and the heart grew very feeble. A mild delirium prevailed at times. The temperature varied, generally about 99° F. She died on October 28, 6.30 A.M.

*Autopsy Protocol* (abbreviated).
(Symmers) Oct. 28th, 2 P.M.

Body 145 cm. in length, of delicate frame and poor nutrition. Veins of neck prominent. Old laparotomy scar in right hypochondrium extending vertically downward to 4 cm. below the umbilicus. No marked external œdema; abdomen very prominent.

On section a moderate amount of subcutaneous fat, muscle tissue pale, moist. Abdomen contains a great deal of straw-colored clear fluid; omentum firmly and extensively attached to parietal part of belly wall corresponding to the scar. The peritoneum is generally thickened. Liver presents in epigastrium and right hypochondrium, 8 cm. below free border of the ribs and 12 cm. below sternum. Diaphragm 4th interspace on the right side, and 5th on the left side. Both pleuræ and the pericardium contain considerable clear serous fluid.

*Heart.*—Hypertrophy of the left ventricle, chronic endocarditis of the mitral valve and cloudy, brown muscle. *Lungs.*—Interstitial pneumonia, emphysema, hydrothorax. *Spleen.*—Very small, pulp prominent dark red. *Kidneys.*—Small, irregularly lobulated, capsule adherent, surface granular, cortex irregular, narrow, markings largely obliterated, fibrous tissue prominent. *Brain.*—Pale, cortex thin. *Stomach and Intestines.*—Show chronic hæmorrhagic inflammation.

*Liver.*—Large, firm; weight, 1080 grams; covered by partly atrophic and partly thickened capsule; marked variation in color, in places pinkish, in others yellow. On section structure for the most part invisible, consistency leathery. Numerous dark yellow pinhead-size dots distributed throughout a pale diffuse, yellowish-white tissue. Near the surface the organ shows streaky, vascular injection, or more roundish distinctly hæmorrhagic areas.

Here again the microscopic sections of the liver show to the naked eye the same irregular or more elongated streaks of pale non-staining areas, interchanging with well preserved tissue, but the very marked and extensive bile imbibition of the affected areas is at once visible either in a more diffuse yellow tinge of the pale spots or as distinct brownish dots and streaks.

The microscopic examination of these sections shows that we have to deal with an exaggerated process of exactly the same type as the former. Here the cells have mostly disappeared, only shadows of them may occasionally be seen; large fat drops occupy the spaces; the remaining reticulum, however, can be made out more frequently. The capillary blood and bile stasis is irregular but pronounced in all affected areas, the latter especially being extreme. The wasting cells are filled with, for the most part, coarse dark yellowish-brown pigment and fat drops, and the disintegrated portions contain bile in large clumps, usually mixed with still well preserved red blood cells. The distribution is general and of equal severity throughout the liver. The localization, although the process frequently can be seen to arise around the central vein, is not confined to a particular area of the liver lobule, and the change is well circumscribed. Free nuclei are common here as in the well preserved portions of liver tissue. This latter shows well marked fatty infiltration and moderate patchy parenchymatous swelling. The portal spaces, much as in Case II, show cellular sclerosis about equally prominent around vessels and bile ducts, and a limited, well confined bile-duct formation. There exists no demarcating inflammatory reaction anywhere within the liver tissue, and no line of demarcation between involved and preserved parts.

*Case IV.*—L. R., colored, male, 65 years old, admitted to City Hospital, December 22, 1904, complaining of asthma and general weakness. He dates this latter condition to a fall from a ladder six or seven years ago, but has suffered from asthmatic attacks more or less all his life. He is a laborer, moderate drinker, and only admits malaria forty years ago. In 1893 he lost his right arm in an accident.

*Physical Examination.*—Patient's breathing is labored, he has difficulty in speaking and a hoarse rattle in his throat. Entire chest emphysematous and barrel-shaped; jugular veins dilated. Rattle of mucus in bronchi, and loud mucous râles everywhere. Abdomen distended, smooth, tense, recti very rigid. Liver border reaches down about 6 or 8 centimeters below the costal margins and in the median line about the same distance, blending with a small area of cardiac dullness. The heart almost covered by emphysematous lungs. Both legs much swollen. Urine, sp. gr., 1.016, very large amount of albumin, no sugar, many hyaline and granular casts, dark amber color.

Clinical diagnosis: Chronic bronchitis, emphysema, chronic myocarditis, and chronic nephritis.

Subsequent history: The patient's condition practically unchanged but for slight remissions. His temperature was usually about 99° F., once or twice rising to 102°. Sensorium dull. He died on February 6, 1905, 12.05 noon.

*Autopsy Protocol* (abbreviated).
(Crowell) Feb. 7th, 11 A.M.

Body 168 cm. long, of poor nutrition and musculature, no external œdema except about the ankles. On section small amount of pale œdematous fat, muscle tissue pale and moist. On opening abdomen large omentum found pulled up over stomach to left hypochondrium, intestine dilated. Liver 4 cm. below free border of ribs on right side and 9 cm. below xyphoid. Gall bladder visible, interlobar septum in median line. Diaphragm stands at the 5th rib on either side. On opening thorax lungs retracted, right pleural cavity contains a liter of turbid yellow fluid, left pleural cavity contains ½ liter. Precordial area large, prominent in left lower half of thorax. Pericardium contains 75 c.c. clear fluid.

*Heart.*—Large, right side engorged, left collapsed, apex formed by left ventricle outside the nipple line. Valves with moderate thickening. Left ventricle large; at apex, involving an area of about 4 to 5 cm. in length, beginning within the muscle and extending to the surface there is a recent anæmic infarct, surrounded by hæmorrhagic infiltration. The rest of heart muscle pale, cloudy, very fibrous; thickness at mitral, 1.5 cm. Larger coronary vessels not obstructed. Wt., 680 grm. *Left Lung.*—Emphysematous and very œdematous, bronchi with much purulent exudate, diffusely reddened. Vessels free. *Right Lung.*—Upper and middle lobes with large, recent hæmorrhagic infarcts. Lower lobe emphysematous and œdematous. *Spleen.*—Small, with scar of an old infarct, generally atrophic; interstitial tissue marked. *Suprarenals* (both).—Atrophic, moderate amount of pigment. *Left Kidney.*—With little perinephric fat. Capsule thin, strips with ease except for localized adhesions. Surface deep red. Cortex moderately contracted, glomeruli prominent; hyalin. Pyramids deep red, indistinct markings. Vessels prominent. *Right Kidney.*—Similar, but with better preserved cortex. Portal structures free. *Liver.*—Rather small, soft, pale, well marked central veins and prominent interlobular spaces, but pale lobules. Gall bladder with four soft yellow stones. *Stomach and Intestines.*—With atrophic, pale mucous membrane. *Brain.*—Moderate œdema, flattened convolutions; thin, pale cortex.

Microscopic sections of the liver show well circumscribed, limited and streaky solution of the liver cells, as described above, leaving a hazy reticulum, with occasional blood stasis, and general fine and coarse bile imbibition of disintegrating liver cells, and accumulation of small fat drops within the dissolving protoplasm. The process follows more frequently the peripheral parts of the lobules, but occurs also irregularly and in streaks around the central veins. The change to normal liver cells is quite abrupt, and there exists slight parenchymatous swelling and fatty infiltration of the uninvolved areas, but never pronounced bile imbibition or blood stasis. The central veins appear unchanged; the portal spaces show moderate fibrous and sometimes hyalin sclerosis around the vessels; a more cellular growth exists around the bile ducts.

These four cases present a change of the same quality, but with some quantitative differences. Common to all is culiar and characteristic multiple and irregular destruc the liver tissue, with more or less pronounced bile an stasis and fatty change, leading grossly to entire loss of ma

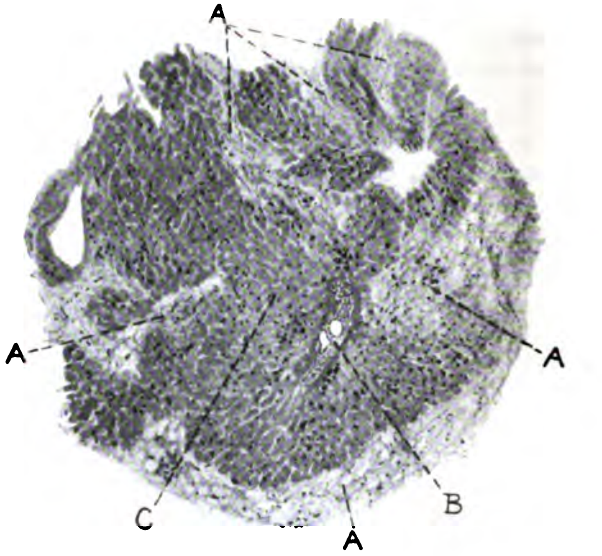

Fig. 1.

leathery consistency, pale yellow color with circumscrib yellow or hæmorrhagic spots and streaks, and an entire inflammatory reaction within the liver tissue, and c portal sclerosis.

It seems certain, as I emphasized in my first report, have here a process that differs essentially from the or observed destruction of parenchymatous cells.[5] It car grouped with the parenchymatous degeneration or nec Virchow's and Weigert's sense, for it does not present th ing with granular disintegration and coagulation of the plasm, with the early loss of well defined cellular outline, ultimate production of a structureless mass of dead prot but, on the contrary, the cell contents show a uniform with the outline well preserved and no particular proce

[5] There can, in my opinion, be no question of a post-mortem cha

as coagulation or granular disintegration, leading to the death of the cell, only a fatty change frequently being associated with the loss of substance, the ability to secrete bile being retained to a late stage.

The change appears too severe, irregular, and circumscribed to belong to the class of simple atrophies, due to quantitative interference with the nutrition of the cells, nor does its morphology correspond to the latter, for, while in atrophy the protoplasm undergoes a gradual loss from periphery to center, so that some parts of the cell at least are preserved, the general fading is here characteristic. The differences between this and other cellular destructions are evident at a glance. No better term could be given to the lesion than solution of cells, cytolysis, and this lesion assumes importance in view of the recent general interest in the question of cytolysis, as also in relation to some of the experimental results. I shall not attempt to discuss this subject fully in this connection. A rich literature has accumulated during the last few years; I intend therefore only to emphasize certain points that seem to touch upon this morphological observation.[6]

The discovery of autolysis which at first was thought to be entirely of a post-mortem nature was soon to assume importance in the explanation of vital processes. Jacoby was able to demonstrate autolysis by finding leucin and tyrosin after ligating off either the whole or parts of the liver, and expressed the opinion that the disintegration in phosphorus poisoning is identical with autolysis. Umber found the same for absolutely fresh exudates,[7] but of especial importance as bearing upon the present observation is that of Conradi, who showed that during the autolysis of organs substances preventing coagulation appear, and these directly antagonize the action of coagulating substances derived from the original organ juice. The are of course especially concerned in coagulation necrosis extensive application of autolysis to the explanation of logical processes has been made by Fr. Müller.[8]

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[6] See F. Umber, "Die klinische pathologische Bedeutung der Autolyse," *Berliner klinische Wochenschrift*, 1903, xl, 185. Recently the influence of osmotic changes has found strong supporters in the explanation of cytolysis. See especially Baumagarten," Ergebnisse d. osmologischen Forschung über Haemolyse," in H. Zickel, *Osmolog. Diagnostik u. Therapie.*

[7] This has been extensively studied lately by Opie, *Journal of Experimental Medicine*, 1905, vii, 316, 759.

Further, according to Jacoby, autolytic ferments are s for the native albumin of the organ from which they are de It is well, therefore, to think of a relation which these e mental chemical observations may have to these anat findings.

Apparently little attention has been paid to the morpho changes incident to autolysis. Waldvogel[9] in some recer tributions has paid some attention to the morphology of mortem liver autolysis, especially to establish that, as far fatty changes in the cells are concerned, great similarity between autolysis and phosphorus poisoning.

The fatty changes[10] in all of our cases were quite prono In such disintegrating liver cells fine or coarse fat granu drops, appeared early within the fading protoplasm, but w definite regard to location. As the cell disappeared, round fat drops occupied the protoplasm, the nucleu pressed to one side, and an accumulation of similar fat was common in the wasted areas. But a marked variat the morphological fatty changes, as well as in the quanti distribution, was visible in all the cases, and even in each so that, while we have to regard them as a constant ass no particular characteristics were detected in this respect. fat in uninvolved liver cells was of the large round drop va usually spoken of as infiltration.

Waldvogel's investigations have also shown a great vari morphological fatty changes in autolysis as well as in phos poisoning. In both, drops, clumps, and granules in large numbers occur, and in autolytic livers a large number of masses staining with osmic acid may even be detected within vessels. Waldvogel further regards the macroscopic color of the liver as to a certain extent at least characteristic of the chemical changes: yellowish white indicating a prevalence of protagon, brown a prevalence of jecorin. Our cases showed a diffuse pale yellow as ground color.

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[8] See also Flexner: "Note on Autolysis in Lobar and Unresolved P nia," *Transactions of the Association of American Physicians*, 1903, xvii

[9] "Autolyse und fettige Degeneration," *Virchow's Archiv*, 1904, cl "Phosphorvergiftung und Autolyse," *Deutsches Archiv. f. klin. Medizi* lxxxii, 437.

[10] In the light of our recent knowledge of fatty degeneration of cells, occurrence of lecithin and protagon under such conditions, it will be of to pay particular attention to these points in future observations of th

It would be of very great interest to compare in detail the various morphological changes in experimental autolytic destruction of cells with our anatomical observations, especially with regard to the occurrence or absence of coagulation necrosis.[11]

Our cases certainly show a cellular destruction unlike that observed in acute yellow atrophy; and none of the associated inflammatory changes of the latter condition are present.

Of particular interest is the bile imbibition of the disintegrating liver cells. It might perhaps be thought that this may stand in causal relation to the lysis of the cells, especially since it has been demonstrated by Salkowsky that the bile possesses such lytic power. Close observation, however, does not bear this out, for not only does the degree of bile imbibition show no relation to the loss of tissue in any of the cases, but there exist also marked individual variations, while the cellular destruction is equally prominent in all four cases.

Investigation shows that the bile is present only in the disintegrating cells, and appears in two distinct types (Figs. 2 and 3): first in the form of fine, small, round, light yellow granules, diffusely occupying the cell, and apparently lying loose on the surface of the disintegrating pale protoplasm. It has all the appearance of a passive invasion. Entirely different is the second, which appears as large, well localized central deposits of bile clumps within preformed vacuoles of the cellular protoplasm. This seems to occur in the better preserved cells, and raises the question whether this form does not present an active secretion of the cell; in that case it would indicate that the destruction of the cells is a gradual one during which cer their functions may be retained to a late stage.[12]

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[11] Such observations are now contemplated in this laboratory.

Of importance seems the fact that all the lesions occu individuals whose general nutrition was lowered, who marked degenerative changes throughout, and who ha exposed for some time to unhealthy modes of living.

Clinically the course was that of an intoxication, if judge from the somewhat meager histories. In three patients delirium prevailed, and in one patient an inc mental dullness.

The extent of the destruction varied quantitatively: th and general jaundice, fatty changes, and the amount a tribution of the portal sclerosis with its bile duct prolifera

It is noteworthy that in a little over a year we have be to collect four well defined cases.[13] While this may parti accounted for by the kind of cases received in an instituti ours, it raises the question whether closer attention wou reveal the lesion more frequently. Cases now classi superficial examination as irregular stasis or parenchy degeneration may, if studied more carefully, be found to to this category.

It seems as if one would have to regard this affection liver as one deserving an independent pathological positio as representing a definite form of cell degeneration. anatomical and experimental investigation will have to mine the clinical and pathological importance and exact p and nature of the lesions.

I desire to express my thanks to Drs. E. Evans, T. C. Ja and N. B. Potter for allowing me the use of the cases a clinical records of them. Dr. B. C. Crowell of the laborat been of great assistance to me during the course of this The drawings are by Mr. K. K. Bosse.

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[12] I have already touched upon the importance of degenerative sec "Der primare Leber Krebs," etc., *Virchow's Archiv*, 1905, clxxx, 499.

[13] Among 275 autopsies from October 13, 1903, to February 7, 190

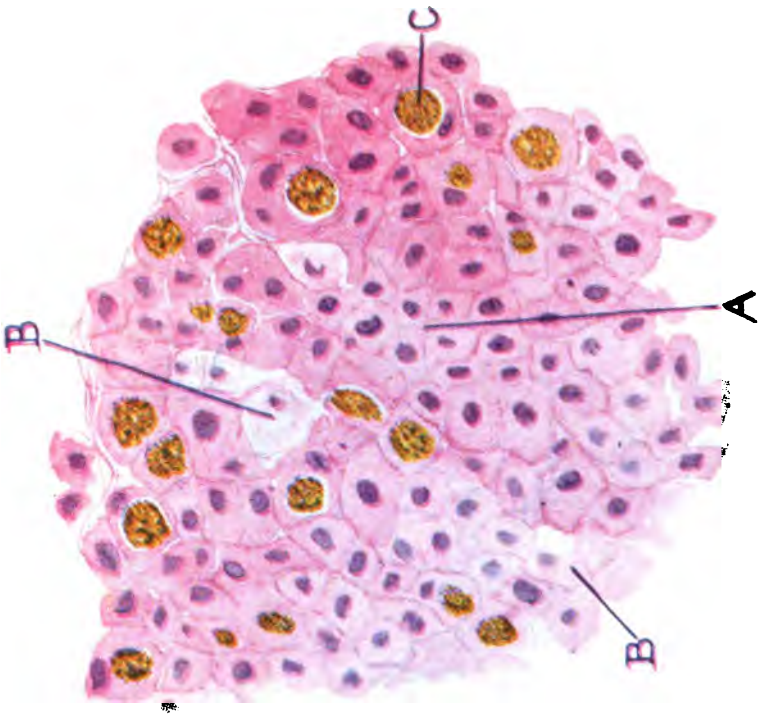

FIG. 3

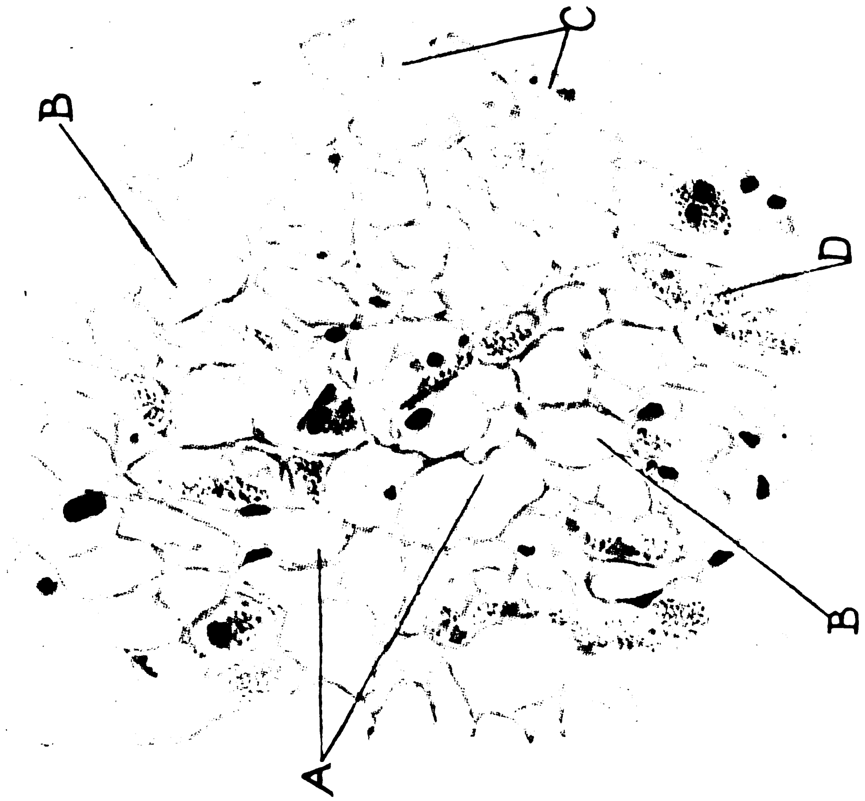

FIG. 2

## EXPLANATION OF FIGURES.

Fig. 1.—In text. From Case I.—Leitz obj. 3, ocular 4. General distribution of lesion.

A. Wasted areas showing remaining reticulum with bile granules and cellular remnants.
B. Portal space.
C. Preserved liver tissue with some bile imbibition.

### PLATE VIII.

Fig. 2.—From Case II.—Leitz obj. 6, ocular 4. Appearance of a wasted area under high magnification. Hæmatoxylin-eosin stain.

A. Completely lost portion with remaining reticulum and nuclear remnants.
B. Remnants of undissolved but fading protoplasm (cell ghosts).
C. Better preserved cells with beginning solution of protoplasms and irregular cellular swelling.
D. Bile imbibition of disintegrating cells in form of fine granules.

Fig. 3.—From Case III.—Leitz obj. 6, ocular 4. Bile in liver cells. Hæmatoxylin-eosin stain.

A. Normal liver cells.
B. Different stages of early cell destruction.
C. Coarse bile granules in preformed vacuoles.

# NEUROMA EMBRYONALE OF THE CHOROID PI OF THE CAT.

By H. S. STEENSLAND, M.D.

*(From the Pathological Laboratory of the College of Medicine, Syracuse Un*

PLATES IX AND X.

The examination of the tumor which I am describi made at the request of Dr. Frank P. Knowlton, who de the physiological features of the animal at the meeting American Physiological Society in Philadelphia, Decembe

In 1904, Verhoeff[1] described a rare tumor (terato-ne arising from the Pars ciliaris retinæ. He regarded it nature hitherto unrecognized and he discussed its relation called Glioma retinæ. The primary and secondary optic v embryonic retina, and vitreous humor and the hyaloid brane were represented in the tumor. Verhoeff though the term "terato-neuroma" would seem to express well the characters of the tumor, indicating as it does a mons growth composed of nervous elements, and that the te the advantage of being applicable to tumors of similar which possibly may arise elsewhere in the nervous syste

I shall describe in this paper a tumor of the cat, pr arising in the choroid plexus, which may belong to the class. In its essential features this tumor appears to re the central nervous system, although in an atypical and a embryonic stage, previous to the differentiation of neur In a personal communication Verhoeff, who has examin tions of my tumor, suggests the name "Neuroma embry as applicable to both tumors.

The tumor in the cat was situated in the fourth ventric the region of its greatest diameter it occupied the left the floor of the ventricle, extending from the median the Corpus restiforme. Anteriorly and posteriorly it lay in the depth of the tissue to the ventral side of the ventricle. It was approximately spherical in form and measured in its greatest diameter nine millimeters. The center of the tumor was converted into a large cyst about which the tumor tissue formed a thin wall. In a cross-section of the tumor and surrounding structures a marked disturbance of the internal relations of the medulla and, to a less extent, of the cerebellum was apparent. This was due to the infiltrative growth of the tumor into the tissue, as proven also by the microscopical examination. The tumor consists of tissue resembling the central nervous system in an early stage of development. It shows numerous lumina, in section circular or elongated in form, corresponding to the embryonic neural canal. An inconspicuous mesenchymal framework is present. There is no mesenchymal capsule.

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[1] *Trans. of the Am. Ophthal. Soc.*, 1904, x, 351.

Microscopical examination of the formalin-hardened tumor was made. The Benda method for staining neuroglia fibrils as employed by Huber[2] was used, except that the chloroform method of embedding in paraffin was substituted. Specimens were also stained with hæmatoxylin and eosin, and by Van Gieson's method.

The entire growth is uniform in character. A condition such as that seen in Fig. 4 suggests an early stage of the neural tube, consisting of a single layer of cells or spongioblasts. The cells are not completely isolated from each other so that the tissue is distinctly syncytial in character. The free ends of the cells project through the Membrana limitans epithelii.[3] In Fig. 2 from another part, a later stage of the nervous system is repres in the tumor. The cell boundaries in this part are stil distinct and the neurospongium of His appears to have fo The dividing nucleus in the internal border zone correspon a germinal cell of His. Along the mesenchyma the proto forms a continuous layer corresponding to the Margo lim This layer is seen in the figure along two sides, but in cases, where the tube is seen in section to be completel rounded with mesenchyma, the picture is even more sugg of the normal neural tube with its embryonic membranes. space separating the two represents, perhaps, the epice lymph space, but it is not to be excluded that this appea may be due to the fixing reagent.

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[2] Huber, "Studies on the Neuroglia," *Am. Jour. Anat.*, 1901, i, 45.

[3] Dr. Verhoeff proposes this name for the membrane described by him ("A Hitherto Undescribed Membrane of the Eye, and its Significance," *Royal London Ophthal. Hosp. Reports*, 1903, xv, 309), and through his generosity I am permitted to introduce it here. He has also published further observations on the membrane ("The Mixed Tumors of the Lachrymal and Salivary Glands," *Jour. of Med. Research*, 1905, xiii, 319). It is described as a fenestrated membrane, occurring also in the ependyma of the central nervous system, and is compared to a wire screen with hexagonal openings, the latter corresponding in size to the cross-sections of the cells. It corresponds, no doubt, to what is frequently described as the Membrana limitans interna or as a cuticular surface. When seen in cross-section, the membrane gives the appearance of a sharp internal margin to the cells. When seen in plane-section its fenestrated structure is

In Fig. 1 a portion of the wall of a tube in a still later st development is represented. A section of a blood-vessel sponding to those which penetrate the normal neural tu this stage is also to be seen. The Margo limitans is deve about the vessel. The Margo limitans is seen especiall developed below the vessel in the figure, and it appears w separated from the vessel wall by a space across which slender strands of tissue.

The nuclei appear large, vesicular, and round or oval in tions. In the tissues fixed in formalin they have a well-ma chromatin network. The nuclear membrane is distinct large nucleoli are present (Figs. 1, 2, 3, and 4). In sec stained with hæmatoxylin and eosin the nucleoli appear refr and translucent (Fig. 1), while with the Benda method stain intensely black (Figs. 2, 3, and 4). The long axes o nuclei are usually arranged radially to the lumina. The t in the sections appears never to reach a stage where mi ceases, and hence mitotic figures are found more or less quently throughout.

Where the tissue shows later stages of development the

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apparent. In the embryonic nervous system the ependymal dots descri Weigert in adult ependyma, where many of them occur in each cell, are ar in pairs, two occurring in each cell on a level with the fenestrated men and in the center of each of its openings.

tions stained by Benda's method show a few neuroglia fibrils, usually at a considerable distance from the lumina. They appear to have the same relation to cells as in normal neuroglia. Nothing corresponding to neuroblasts is seen. Thus a differentiation leading to the formation of neuroglia only, takes place in certain regions.

In serial sections independent lumina can be followed throughout their entire extent. One of the smaller lumina (Fig. 4) is eight microns in diameter measured from the Membrana fenestrata epithelii. The latter membrane is well defined and is seen as a sharp line. The appearance of processes passing out from the membrane between the cells results from the screen-like structure of the membrane. The protoplasm of the spongioblasts appears to project as a continuous mass through the membrane into the lumen. This lumen is evident in only three sections of the series.

It may be that the origin of the lumina is the same as the origin of the lumen in a solid anlage of the nervous system, as in the teleostei. Lumina of intermediate sizes between the smallest and largest are encountered.

Verhoeff speaks in the following way of the possible part played by the Membrana fenestrata epithelii in the development of the lumina in the so-called Glioma retinæ: "The structure of the membrane in the rosettes explains why the latter assume their peculiar form, for it is evident that if a few cells became bound together by such a membrane, and they were otherwise unhampered in their growth, they would be compelled to form a more or less spherical body. If the free ends of the cell groups did not finally meet, a spiral figure would result, and just such spirals are frequently seen. The writer has also found single cells with a membrane on one end, as well as curved groups of several cells which were obviously the beginnings of rosettes. This explains very well why tumor cells are often seen within the cavity of rosettes, evidently being enclosed during the formation of the latter." This method of formation would explain the presence of tumor cells in the lumina in the present case also, but the spiral figures are not met with. Ribbert, in

describing the lumina sometimes occurring in gliomat
that some lumina appear to be surrounded with radi
ranged cells in only a part of their circumference.[4] The
ance would seem sometimes at least to result from irregu
in the form of the lumina. As a result of this the epe
layer is cut tangentially in a part of the circumference,
does not present its best known appearance. In my ca
sibly, the membrane has had a more or less spherical arran
from the time of its origin, the cells in the interior gr
undergoing degeneration and disappearing, but the me
formation of the lumina was not determined.

In Fig. 2 the Membrana fenestrata epithelii is seen to
defined about a considerable portion of the lumen. P
of the cells project through it into the lumen. Tum
are present in the lumen, a condition which is seen freq
The protoplasm of these cells appears to have undergone
eration, staining relatively deeply. The nuclei usually a
or less indistinct. The protoplasm frequently is continuo
the processes of the cells extending into the lumen. The
tion, such as that presented in this figure, suggests that
the internal border zone, instead of migrating peripherall
sometimes migrate into the lumina, and then, on account
favorable nutritive conditions, gradually undergo degene

In sections tangential to the lumina and passing betw
Membrana fenestrata epithelii and the nuclei a further st
the form of the "columnar cells" may be made. The t
seen to consist of a syncytium in which there are large
(Fig. 3). These spaces correspond to those which, in s
passing through the center of lumina, appear to separat
vidual "columnar cells" from each other.

The tissue appears to be arranged symmetrically ab
lumina in every direction. Nothing corresponding to the
ventral axis of the developing nervous system is seen
elongated form of some of the lumina suggests a represe
of the antero-posterior axis. Sometimes the tubes are fo
a way resembling the folding of the normal neural tube

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[4] Ribbert, *Geschwulstlehre*, 1904, 336.

development of the various territories of the central nervous system.

In sections tangential to the lumina a study of the Membrana fenestrata epithelii may be made. Pictures corresponding exactly, as far as the membrane and ependymal dots are concerned, to those given in the description of the tumor of the Pars ciliaris, are seen. Where the section passes near the periphery of the lumen the membrane is seen both in cross- and plane-section. The membrane appears to be present in connection with the smallest lumina.

The method of growth of the mesenchymal framework is not evident. No active proliferative process is seen in it. The larger vessels are surrounded by a cellular adventitial sheath which is closely applied to the wall of the vessel and frequently is separated by a wide space from the surrounding epithelium. Often, however, the epithelium is closely applied to the mesenchymal tissue, in which case no evidence of the perivascular space exists.

*Origin of the Tumor.*—In the region of the fourth ventricle the cells which would seem most likely to possess the potentiality for the formation of the entire nervous system are the epithelial cells lining the choroid plexus. Morphologically, also, they correspond most nearly to the original epithelial cells of the medullary plate and the neural tube. Such an origin would correspond to the origin of the tumor of the Pars ciliaris retinæ, from the unpigmented epithelium of the retina. In sections the epithelium of the choroid plexus may be traced into direct continuity with tumor cells, and transitional forms between the two appear to occur. Pictures are seen corresponding to Fig. 2 in Verhoeff's description, which shows the tumor cells in continuity with the unpigmented epithelium.

In the case of the tumor of the Pars ciliaris it seems probable that the original process was one of resumption by the unpigmented epithelium of properties leading to the formation of the primary and secondary optic vesicles (Verhoeff's Figs. 2 and 8). The condition of the cells in these figures corresponds more or less to those present in my Fig. 4. The lumina in Verhoeff's

tumor arise as the result of a convoluted papillary outg
the epithelium and the underlying stroma. The "sec
of convolution," representing the early embryonic ret
hoeff's Fig. 6), corresponds most nearly to that shown by r

In the case of the tumor of the choroid plexus of th
epithelium lining the plexus appears to have resumed,
atypically, the properties of the epithelium of the e
neural tube.

It may be objected that if the tumor is to be regar
neuroma there should be evidence of the formation o
blasts. But the tissue may be regarded as in a stage of
ment anterior to the appearance of these cells. Moreov
are territories in the lining of the neural tube aside from
giving rise to the choroid plexus, that give origin only to r
and not at all to nervous elements. This is true, for
of the territories from which the optic nerves origina
epithelial cells give rise simply to the neuroglia framewo
nerves which supports the fibers of the nerve cells origi
another level. Yet these cells probably possess the pot
for the formation of neuroblasts. Perhaps some tu
scribed as ependymal gliomata belong in the same class
tumor.

For the drawings of Figs. 2, 3, and 4 I am indebte
Francis A. Hulst.

## EXPLANATION OF PLATES IX AND X.

In all the figures the outlines were drawn with the aid of the Ab
lucida (Leitz Obj. 7, Oc. 5), and the detail is added with higher ma
(Leitz Obj. $\frac{1}{12}$, Oc. 5). The drawings, except Fig. 1, are all slightl

Fig. 1.—A part of the wall of a relatively large tube. Hæmato
eosin staining.

Fig. 2.—Medium size tube showing the Margo limitans on two si
appearance resembles the cross-section of the embryonic neural t
stroma is shown to the right and below. Benda's neuroglia fibril sta

Fig. 3.—Section tangential to a lumen between the Membrana
epithelii and the nuclei. The relatively deeply staining area of pro
the center of the figure is found in neighboring sections of the se
associated with a mitotic nucleus. Small amount of stroma shown
right-hand corner. Benda's neuroglia fibril stain.

Fig. 4.—Section through the center of one of smaller tubes. The a
similar to that of the neural tube consisting of one layer of cells. Ben

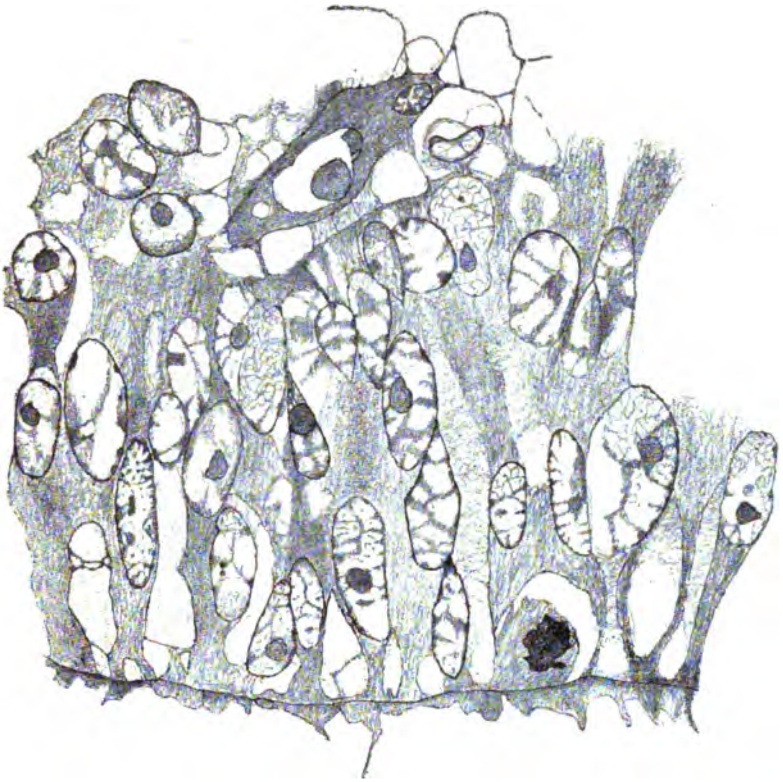
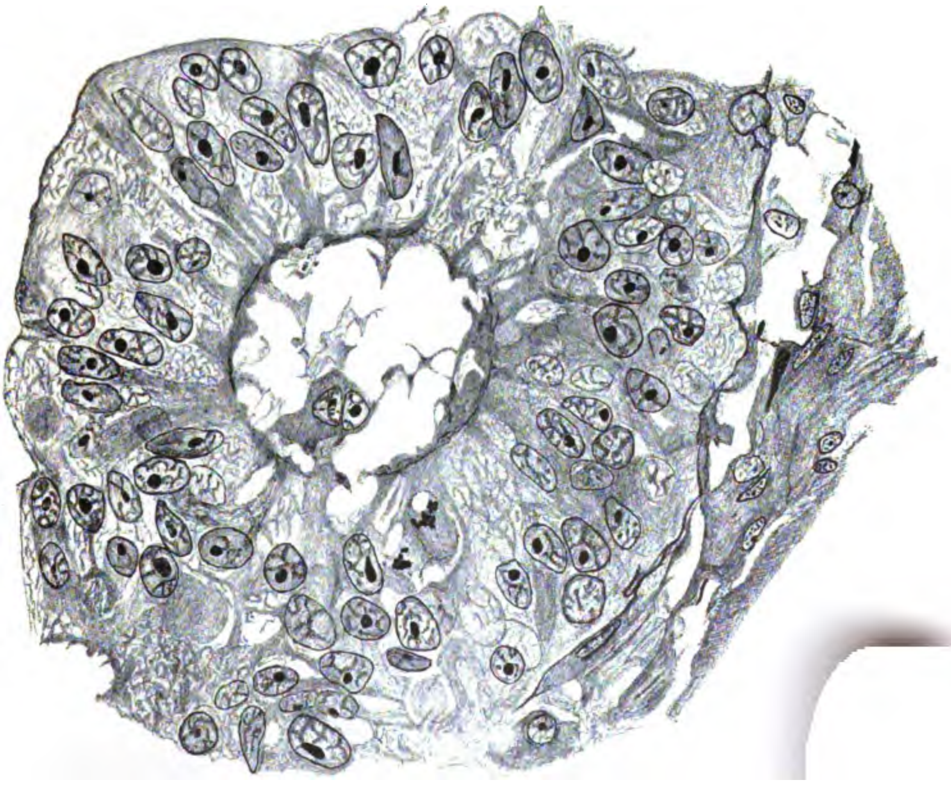


FIG. I



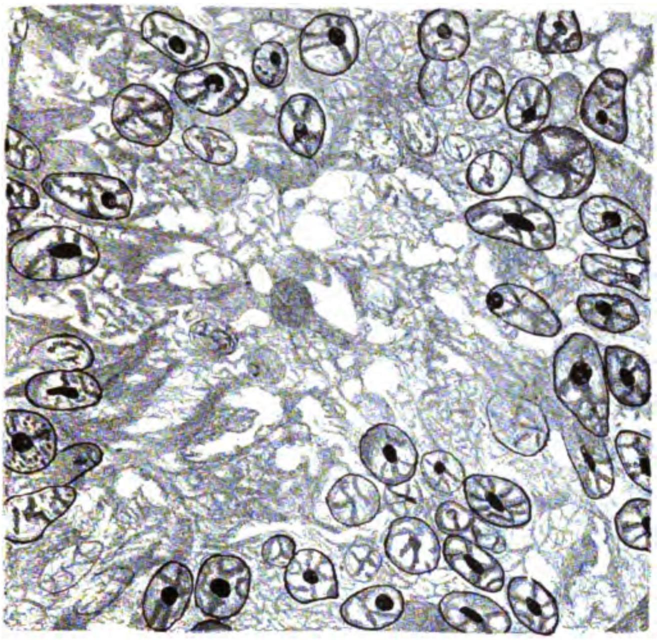


FIG. 3

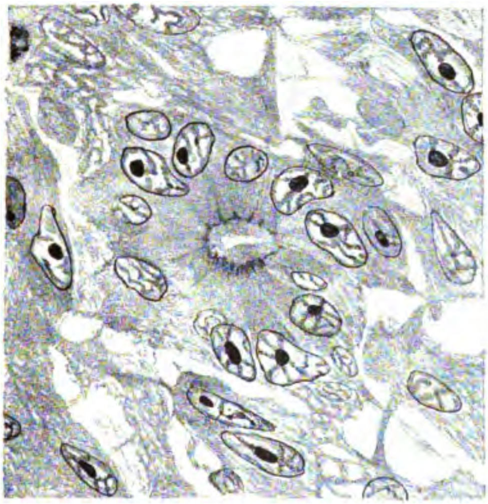


FIG. 4

# STUDIES ON THE TOXICITY OF BILE.

## II.

## THE TOXIC EFFECTS OF BILE UPON THE CENTRAL NERVOUS SYSTEM AND THE ELIMINATION OF STRYCHNINE THROUGH THE BILE IN NEPHRECTOMIZED ANIMALS.

By S. J. MELTZER and WILLIAM SALANT.

(*From the Rockefeller Institute for Medical Research, New York.*)

The occurrence of severe nervous manifestations in the course of diseases in which icterus is a predominant symptom gave rise at an early period to experimental investigations of the effect of bile upon the animal organism. During the first part of the last century the methods employed in these investigations consisted mainly in intravenous injections of ox bile obtained from the gall bladder. As in most of the experiments the injections caused the death of the animal, it was ascribed to the toxic effect of the bile. During the forties of the last century, however, the view gained ground that death following intravenous bile injections was not due to the toxicity of the bile, but to impurities in the fluid used which give rise to the formation of capillary thrombi in vital nerve centres. Thus Bouisson reported that animals which received filtered bile survived the intravenous injections without manifesting any important nervous symptoms. This result could not have failed to exert a deciding influence upon contemporary opinion, and we need not therefore be surprised to find Henle[1] making the statement that the widespread view of the toxicity of bile was an unwarranted assumption. However, after the discovery by Strecker[2] of bile acids, numerous investigations were carried out with them, and any question of accidental causation of thrombosis could therefore no longer

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[1] Henle, *Handbuch der rationellen Pathologie*, 1847.

[2] A. Strecker, *Habilitationsschrift*. Giessen, 1848.

enter seriously into the discussion of the results. Th
extensive study of the toxicity of bile salts was made l
Dusch.[3] And among the subsequent investigators we fin
well-known names as Frerichs, Leyden, Kühne, Traube, I
Seyler, Landois, and many others. The last investigat
this subject, so far at least as the effect upon the nervous s
is concerned, was carried out some fourteen years ago
Kobert's direction by D. Rywosch.[4] It was a compreh
painstaking piece of work which has left its impression u
later writings on the subject of the toxicity of bile.

The present status of the views entertained concerning the toxicit
may be described as follows: All agree that bile is a very toxic substa
hæmolyzes red blood corpuscles and dissolves white blood corpuscles a
tissue cells; it retards and stops the heart-beat, lowers the blood press
is capable of affecting the structure of nerve and muscle, etc. Regar
effect upon the central nervous system, there is unanimity of opinion t
produces depression; most of the investigators describe stupor and w
of the animals as the predominant symptoms following the intraveno
tion of bile salts in mammals, and coma and paralysis following their i
into the lymph-sacs of frogs. A few investigators have also observ
sionally the occurrence of convulsions. von Dusch [5] describes three
ments in which the animals died in convulsions a few minutes after r
intravenous injections either of filtered bile or of sodium cholate.
observed similar effects in two experiments in which 7 c.c. of a 5 per ce
tion of sodium cholate were injected into the jugular vein. Leyd
scribed a few experiments in which convulsions appeared after injectio
salts into the jugular vein; in one case the convulsions appeared imm
after injection of one c.c. of a 10 per cent. solution of sodium glycochol
the vast literature on the studies of bile there are scattered records
more experiments in which convulsions occurred among the effects of
jection of bile. However, even Leyden, who more than any other au
sisted upon the appearance of convulsions after intoxication with bil
that after all it is a rare occurrence. We [8] have ourselves stated in a
paper that in most experiments the convulsions set in soon after the intr
injection of bile or bile salts, and have expressed the view that they wer
terminal manifestations due to heart failure produced by too rapid inj
the solutions. In a few exceptional cases convulsions are recorded

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[3] von Dusch, *Habilitationsschrift.* Leipzig, 1854.

[4] D. Rywosch, *Inaugural Dissertation.* Dorpat, 1891.

[5] von Dusch, l. c.

[6] Röhrig, *Dissertation.* Würzburg, 1863.

[7] Leyden, *Beiträge zur Pathologie des Icterus.* Berlin, 1866.

[8] Meltzer and Salant, *Journal of Experimental Medicine*, 1905, vii, 2

developed a day or two after subcutaneous or intraperitoneal injections. Since, however, in these instances the bile or bile salts tend to produce extensive local necrosis, the convulsions are not necessarily to be regarded as due directly to the bile; they may have been caused by absorption of toxic products from the necrotic areas, or the result of a terminal infection. In frogs the toxic symptoms appear so soon after the injection of bile or bile salts into the lymph-sacs as to be ascribed directly to bile intoxication. Leyden,[9] Loewit,[10] and Rywosch [11] tested bile salts upon frogs, and report that coma and paralysis are the only effects which they have observed. Rywosch indeed asserts that in all his experiments, in which mammals were also included, he never saw anything but a depressing effect. It was this last statement which caused the more recent writers on diseases of the liver to abandon the old theory of cholæmia as due to bile absorption in acute yellow atrophy of the liver or of any other form of *icterus gravis*. The symptoms are sometimes of a depressing character, as in coma, etc., and sometimes manifestations of increased excitability, as in convulsions or excitation mania. On account of the occurrence of the latter symptoms, Stadelman [12] and others insisted that bile absorption could not be the cause, and they argued that bile causes only coma and similar symptoms of a depressing nature, but never convulsions.[13]

To sum up briefly, it may be said that bile, according to the present almost universally accepted view, contains a toxic element which is capable of producing nervous depression, coma, and paralysis, but does not contain any element which can produce excitation and convulsions.

Our experiments have consisted in the injection of bile and bile salts into animals. We have employed chiefly rabbits' bile, although bile from the ox, dog, and guinea-pig was also used. In addition to this, experiments with glycocholate and taurocholate of sodium were made. The frog was chiefly employed for the injections, which were made into the dorsal or ventral lymph-sacs. There are many reasons for selecting frogs for the experi-

[9] Leyden, l. c.

[10] Loewit, *Zeitschrift für Heilkunde*, 1881, ii, 459.

[11] Rywosch, l. c.

[12] Stadelman, *Ueber Icterus*. Stuttgart, 1891.

[13] The recent observation by Biedl and Kraus [14] that subdural injections of bile cause convulsions can hardly be taken as a valid proof that bile contains a tetanizing element, since even minute doses of such comparatively innocent substances as methylene blue, which by intravenous injection are almost harmless to the animal organism, can produce convulsions when injected subdurally or intracerebrally.[15]

[14] Biedl and Kraus, *Centrabl. f. klin. Med.*, 1898, p. 1185.

[15] Bruno, *Deutsche med. Wochensch.*, 1899, xxv, 369.

ments, for, from what we have stated above, it is evider mammals are not very suitable for them. When in ma the injections are made intravenously, the rapid reactions often follow are due to the effect of the bile upon the hea not to a primary effect upon the nervous system. When, other hand, the injections are made into the peritoneum the reactions sometimes set in so late as again to permit of as to whether they are or are not primary toxic effects. case with frogs is different. The reactions which follow t jections of bile into the lymph-sacs set in early enough to directly to the bile intoxication; nor can there be any sus that the nervous manifestations are secondary to some di ance in the circulatory or respiratory systems.

As the main title of this paper indicates, the chief part research was devoted to a study of the toxic effects of bile the central nervous system. This, however, was not the p consideration which started us on this long investigation indeed, we were in quest of a solution to a problem whic sented itself to us in the course of our previous resea Before the latter could be attacked, we became confronte the question of the normal toxicity of bile, in the study of new thread we arrived at results which shed light on th problem, while, at the same time, it assisted in elucidati original problem. And herein will be found the chief reas using rabbits' bile upon frogs. In presenting our results it be natural to follow the tortuous path by which we gradua rived at them. We shall therefore begin with the statem the original problem which started us on this research.

It is an established fact that strychnine when introduce the body by any path leaves it entirely through the kidneys. have, however, found that after double nephrectomy, t three times the fatal dose of strychnine can be injected w causing intoxication, provided each single quantity injec less than the minimum toxic dose, and the intervals betwe injections are not too short; that is, it appeared that aft phrectomy there is no cumulative effect of the strychnine

[16] Meltzer and Salant, *Journal of Experimental Medicine*, 1901–1902,

blood. One of the possible explanations for this remarkable condition lies in the assumption that after nephrectomy the process of elimination is assumed by another organ. The gastrointestinal canal suggested itself to us as this channel,[17] but we failed to detect strychnine in it. Later, [18] it was found that the methods at hand for detecting traces of strychnine make it difficult to discover small quantities of it in the intestinal canal. Hence, in the further pursuance of the question of vicarious elimination, we turned our attention to the bile through which, it is claimed, poisons are normally eliminated from the body. Our problem therefore was to determine whether, after nephrectomy, strychnine is eliminated through the bile. As our previous experiments were made on rabbits, it was necessary therefore to make the new ones on the same animal. Hence our reason for using rabbits' bile in this investigation. Furthermore, as the chemical method for separating strychnine from bile is very tedious, and would become quite unmanageable if, as in our plan, a large number of experiments were to be performed, we resorted to the biological test—i. e., the injection of bile supposed to contain strychnine into frogs in order to produce the usual well-known strychnine convulsions.

In attempting to solve this problem several series of experiments were made. In one series the rabbits which were nephrectomized had their common bile duct ligated. After recovery from the operation the animals were given several subminimum doses of strychnine. The animals were studied first with reference to the amount of strychnine they could stand without suffering a convulsion, and second, after the death, in that the bile from the gall bladder and the dilated duct was injected into frogs. In another series of experiments a cannula was tied in the common duct of the nephrectomized rabbits, and, after injecting subcutaneously subminimum doses of strychnine, the collected bile was tested on frogs. The following is a brief *résumé* of these experiments.

Series I.—*Both kidneys removed, the common bile duct ligated,*

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[17] Salant, *American Medicine*, 1902, iv, 293.

[18] Salant, *Journal of Medical Research*, 1904, xii, 41.

*and, subsequently, subminimum doses of strychnine inje*
*varying intervals.*

This series included seven rabbits. Four showed no signs of a cun
effect, although the sum total of the several doses of strychnine in each
exceeded by far the minimum fatal dose (about 0.6 milligramme p
One animal lived 74 hours and received during that time nearly
grammes per kilo without showing either convulsions or hyperæsthe
died under the symptoms of exhaustion.

The behavior of these four animals was identical with that of nephrec
rabbits in which the common duct was not ligated.

Of the remaining animals, one received within twelve hours about o
gramme per kilo of strychnine without showing any effects. Three hou
however, when it was again given about 0.2 milligramme per kilo, the
had a tetanus and died in a few minutes. The course in this experim
not differ materially from that which sometimes occurred in our
experiments on nephrectomized animals without ligation of the duc
received strychnine in refractive doses.

The remaining two animals, however, died in violent convulsions tw
after receiving only 0.3 milligramme of strychnine per kilo, a dose whi
a rule, non-toxic even for normal animals.

These observations, then, did not permit of any positiv
clusions being drawn one way or the other. In the majo
the animals the closing of this additional eliminating pa
not increase in the slightest the cumulative effect
poison within the blood, a fact which would speak again
elimination of the strychnine through the bile. On the
hand, in two experiments, ligation of the bile duct did pr
a rather striking aggravating effect upon the injection of a
subminimum dose of strychnine. Furthermore, we had t
into consideration that the absence of strychnine sympto
the majority of these animals could not be taken as an ab
proof of the absence of strychnine in the bile, because i
possible that the refractive doses of strychnine were elimi
from the blood by, and accumulated largely in, the bile
the gall bladder, but through the abnormal state of the bl
under these conditions the strychnine was prevented from
re-absorbed into the circulation, which would account f
absence of convulsions in most of the experiments.

In the latter case we would expect that the bile of animals when injected into frogs would produce symptoms of strychnine poisoning. This view was tested. Bile was taken from the gall bladder and the dilated bile duct from four rabbits of this series. The quantities were of course small and only a few experiments could therefore be made. The few results were as follows:

One c.c. of the bile injected into a medium-sized frog caused coma in one hour; and ½ c.c. caused in a small frog complete coma and paralysis half an hour after injection. As to the remainder, it can be briefly stated that none of the frogs which received smaller or larger doses of the bile showed convulsions or hyperæsthesia.

These few observations did not indicate that the bile contained strychnine, at least not in such quantity as to set up characteristic symptoms in frogs.

Regarding the preceding experiments it may be said that they were capable of providing positive proof only if all the animals had manifested strychnine poisoning, or in other words if the bile of the animals had contained strychnine we should have had evidence that after nephrectomy strychnine is eliminated in this secretion. However, the failure of these experiments to bring forward this proof can not be interpreted as disproof, namely, that the biliary organs do not assume the function of elimination of strychnine after nephrectomy; for, just as we have assumed that after removal of the kidneys another organ, the liver, takes over the function of elimination, we may now assume that after nephrectomy and ligation of the common duct still another organ assumes the work of elimination of strychnine; whence the failure of the above experiments to show that the bile contained strychnine. We expected to obtain more satisfactory evidence regarding this assumption from the following set of experiments.

Series II.—*Rabbits were nephrectomized and a cannula placed in the bile duct; strychnine in subminimum doses injected, and the bile collected and introduced into frogs.*

We made six such experiments. In five animals some bile was collected before the strychnine injections were given. This bile was also injected into frogs which served as controls. All the frogs receiving bile from the ante-strychnine period responded with coma and all but one became paraly died. None of the frogs showed tetanus, rigidity, or convulsions, and one showed even no hyperæsthesia. One showed marked hyperæsthe soon also passed into coma and paralysis.

The frogs which received the bile from the strychnine period pres different picture. The bile from one rabbit which received within si 0.7 milligramme of strychnine nitrate caused in three frogs tetanus and twenty minutes. The bile from another rabbit which received 2 millig of strychnine in forty-eight hours produced tetanus in two frogs, hyperæsthesia in one, and had no effect in another; in the last case a bile as large as three c.c. was given. Two rabbits were given about o gramme of strychnine; the bile of one produced tetanus in one, marked æsthesia in another, and coma and paralysis in a third frog. The bile of t produced late rigidity and convulsions in one and coma in another frog. remaining two rabbits, one received 0.5 milligramme of strychnine in dose; the bile of this animal caused in one frog hyperæsthesia which terr in coma, and in another it produced only coma. The same effect from the bile of the last rabbit, which received a single dose of strychn amount is not noted in the protocols).

The bile (strychnine period) from the rabbits of this produced distinct strychnine symptoms (hyperæsthesia, e nearly all the frogs, while distinct tetanic attacks were pro by the bile of rabbits which received at least toxic doses. paring these results with those obtained with the bile fro ante-strychnine period of the same series, the conclusion to be justified that in these animals some of the strychn least was eliminated through the bile.

New questions now arose. In the first place, the question whether the presence of strychnine in the bile of this se animals represents a vicarious process only, and whet normal animals which receive strychnine the bile does no as a rule, eliminate part of the poison; and in the second, w the bile of nephrectomized animals, not receiving stryc does not contain a tetanizing element of metabolic origin, may have caused the hyperæsthesia and convulsions des in the last series of experiments. These questions were tes short series of experiments.

Series III.—*Bile collected from a cannula in the commo of normal rabbits before and after the injection of strychni tested on frogs.*

Only three such experiments were made. One animal received 0.9 milligramme of strychnine. Two c.c. of the ante-strychnine bile of this animal gave coma which developed gradually within one hour. Two c.c. of the bile of the strychnine period gave marked hyperæsthesia, and the addition of two c.c. more of the bile brought out immediately a violent tetanus which passed over rapidly into coma and paralysis.

In the second experiment the rabbit received one milligramme of strychnine. Two c.c. of the ante-strychnine bile gave coma as before, whereas the same amount of bile of the strychnine period gave pronounced hyperæsthesia, which after an hour passed over suddenly into coma (a very brief period of convulsions might have been overlooked). In both the experiments the urine collected after the administration of strychnine was also tested; it was almost without effect.

The third animal received 1.5 milligrammes of strychnine. Two c.c. of the bile (before and after strychnine injection) produced no effect. Three c.c of the bile of the strychnine period produced coma, while three c.c. of the bile of the ante-strychnine period produced rigidity of the frog. (The urine of this animal caused increased reflexes in frogs.)

Hence we have among this series two experiments in which the bile from non-nephrectomized rabbits, after injection of strychnine, produced marked hyperæsthesia and even a convulsion, while in the third experiment the normal bile of the rabbit (before receiving strychnine) produced symptoms distinctly similar to those of strychnine poisoning.

Series IV.—*Bile collected from the common duct of nephrectomized rabbits and tested on frogs.*

We had five such experiments. In one even three c.c. of the bile produced no effect upon the frogs, while three and a half and five c.c. brought on prolonged tetanic attacks. In another experiment three c.c. had no effect, and five c.c. produced hyperæsthesia, but neither convulsions nor coma. In a third experiment five c.c. brought on hyperæsthesia and stupor. Of the bile of the fourth experiment three c.c. had no effect, but five c.c. brought on coma and paralysis. Finally, in the fifth experiment two c.c. of the bile produced coma and paralysis.

In this series, with entire absence of strychnine, marked hyperæsthesia was a frequent phenomenon, and distinct tetanic attacks occurred. On the other hand, coma and paralysis were seen only in two instances and in one instance only after injecting five c.c. of the bile.

A remarkable fact of this series is that in nearly all of the experiments as large doses of bile as three c.c. produced no effect at all.

The control bile of all the previous experiments (except one) produced with two c.c., and sometimes even with one c.c., distinct coma and paralysis. The ante-strychnine bile in the second series was also obtained from nephrectomized rabbits and is therefore comparable with the bile of the last series in every respect except one, that it was obtained at the very beginning of the flow of bile from the cannula. This latter fact might indeed be an important feature and perhaps explain the absence of hyperæsthetic and convulsive effects from the bile of the ante-strychnine period of the second series. We may, perhaps, assume that the hypothetical tetanic element, which normally is eliminated through the urine and after nephrectomy finds its way out of the body through the bile, appears in the bile in effective doses only a time after the removal of the kidneys —that is, that the vicariation develops only gradually. The bile of the fourth series was collected during a long period, sometimes extending over two days and longer, after the nephrectomy, whereas the bile of the ante-strychnine period of the second series was collected only an hour or two immediately following the nephrectomy. Hence, the absence of a tetanic element in the one and its frequent occurrence in the other bile.

However this explanation may account satisfactorily for the production of hyperæsthesia and convulsions in the experiments with the bile of the fourth series, it does not explain the remarkable reduction in toxicity. What could the factor be that caused the surprising reductions in the elements producing coma and paralysis in the bile of the fourth series so that even such quantities as three c.c. had no effect?

Could the presence of a tetanic element in the bile, be it strychnine or some catabolic product, influence the activity of the elements producing coma and paralysis normally present in the bile so as to neutralize to some degree the depressing effect?

Before this question could be properly disposed of, a more fundamental proposition confronted us and had to be studied, namely, as to what the actual effect of the bile collected for a longer period from the common duct of a normal animal is. In the preceding series we had not yet dealt with this question. The control bile of the third series, which was a normal bile, was collected only for a short period and soon after the introduction of the cannula into the bile duct, in which instances the possible after-effect of the anæsthesia had to come into consideration. But even in this short series the results were not uniform. The control bile of the three experiments produced coma and paralysis in two and rigidity in one animal.

In reviewing the literature of this subject we learned that the opinion now generally held is that bile produces coma and paralysis. It would seem as if only three investigators experimented upon frogs. Leyden made injections in three, and Rywosch in four frogs, while Loewit, who probably made many injections, states his general conclusions only. The three investigators employed chiefly biliary salts, or when bile was used it was ox bile obtained from the gall bladder. It has been generally assumed that bile obtained from the gall bladder is more poisonous than that obtained from the common duct. It is therefore evident that there has been as yet no information whatsoever secured as to the behavior of bile collected from the common duct of normal rabbits. We have studied this subject by means of a large number of experiments, and will now proceed to report briefly the results obtained from them and from others made with stagnant bile from the gall bladder and the dilated, ligated ducts of otherwise normal rabbits.

Series V.—*Bile of normal rabbits.* a. *Bile from the gall bladder or from the dilated, ligated duct.* b. *Bile collected from a cannula in the common duct.*

*a.* We made only a moderate number of these experiments. The results were as follows. As small quantities as one-half c.c. produced no immediate effect, but most of the animals were found dead the next day. One c.c. produced, as a rule, coma and paralysis an hour or two after injection. In two instances even one and a half c.c. produced only a moderate effect which set in late. (It seemed that injections into the ventral brought out the effect more promptly than into the dorsal lymph-sac. Possibly the abdominal pressure in the normal posture acts like massage and hastens absorption.) In no case was any increase of reflexes, or convulsions, or rigidity observed.

*b.* As a rule large quantities of bile were collected in this manner, sometimes more than 100 c.c., and thus experiments could be performed upon many

frogs with the bile obtained from one animal. The data which we giv
gathered from experiments made with bile of nine different rabbits. In
of the numerous experiments the employment of the normal bile was a
some experiment in which the normal bile served as a control for com
with the effect of bile of some other kind.

The chief result of this series of experiments is the esta
ment of the fact that the bile of some rabbits can produc
tinct hyperæsthesia and tetanic attacks. Whether the b
moderately or strongly toxic, and whether the predomin
toxic effect is depressing, as in coma and paralysis, or exc
as in hyperæsthesia and convulsions, seemed to depend gr
upon the individuality of the animals. Our experience
these animals and with other experiments in the course of
research, led us to feel justified in dividing rabbits into
groups according to their individualities as above indicated.

The bile of one of the groups caused coma and paralys
sentially. Two c.c. would, as a rule, produce these symp
within a few hours, and the larger the quantity injecte
sooner would they set in and the more complete would the
Quantities less than this but above one c.c. produced durin
eight or ten hours of observation scarcely any perceptible e
but on the next day or the third day the animals woul
found dead. One c.c. of bile was almost without action.
bile of these animals produced neither hyperæsthesia nor
vulsions no matter what the amount given. We wish to
phasize that the bile of this group of animals produces
and paralysis but never hyperæsthesia and convulsions.

In a second group of animals bile in quantities of two c
even two and a half c.c. produced no effect whatever. Thre
of bile caused sooner or later more or less marked hyperæst
from which the majority of the frogs recovered. Doses o
c.c. produced symptoms of coma and paralysis more or
distinct. As the doses were increased, the symptoms inten
and appeared the sooner. These animals all succumbed.
main characteristic of this bile is that small doses pro
hyperæsthesia and larger ones coma and paralysis. In e
tional cases coma would be preceded by a convulsive atta

short duration and easily overlooked. On this account we may have missed certain of their manifestations.

The bile of the third group in doses of from two to three c.c. produced at most no effect. Three and a half c.c. or more produced marked hyperæsthesia passing into clonic or tonic convulsions. Sometimes a violent tetanus would set in two or three hours after injection of the bile, and at other times the tetanic effect appeared the next day, or only after prolonged handling of the frogs; it tended to be of a pronounced tonic character, the animal remaining uninterruptedly rigid for two minutes or longer. Again the tetanic effect appeared only after such large doses as six, seven, or more c.c. Some frogs survive mild tetanic attacks and return to a good condition. Those which had violent or prolonged tetanic attacks did not, as a rule, recover, but behaved as if poisoned with a toxic or fatal dose of strychnine. None of the frogs which received bile of this group of animals ever showed coma and paralysis.

The bile of the first or coma-producing group was toxic in small doses; the bile of the third or convulsive group exerted a toxic effect only in larger doses; the bile of the intermediate group, producing hyperæsthesia in smaller, and coma in larger doses, possessed only moderate toxicity.

In no instance did we encounter a bile which in small doses caused coma and in larger ones convulsions or hyperæsthesia.

These experiments established beyond doubt that the bile of some rabbits contains a pronounced tetanic element. In certain specimens of bile the tetanic constituent was the only noticeable toxic agent, in others the depressing toxic element existed in moderate amount, the tetanic effect manifesting itself only by hyperæsthesia and in those doses which did not exert a depressing effect. In one kind of bile only could no evidence of the presence of a tetanic element be discovered, namely, in that which in small doses exerted a strong depressive action leading to coma and paralysis.

The question which now arose was this: Does the bile which produces only coma and paralysis actually contain no tetanic element? From the behavior of the bile of the intermediate group we had reason to believe that the element which pro coma and paralysis, when present in sufficient strengt capable of suppressing the manifestations of the tetanic stituent. It is therefore conceivable that the very depre bile of the first group contains a tetanic body, the manifest of the action of which is rendered impossible because of th of the suppressive action of the depressing element.

In following out this possibility we succeeded in demonst the presence of a tetanic element in the bile of all the ra studied.

We had observed that a medium dose of bile of the inter ate group was capable of bringing out hyperæsthesia alth the bile surely contained a certain amount of the depre element. The reason for this result apparently rested o fact that in this dose of bile, which was subminimum fo depressing effect, the mutual relations between the tetani the depressing substances were in favor of the tetanic We now thought that by artificially supporting by strychni ineffective doses the tetanic element, when present in th in subminimum doses, it might also be possible to develo hyperæsthesia and perhaps even the convulsions.

In a previous part of this paper we were led to consider wh strychnine or a tetanic substance of catabolic origin migh be capable to some extent of overcoming or neutralizin depressing effect of the coma and paralytic elements. Stimu by these questions, we carried out several series of experi in which the effects of bile supported by additional injectio strychnine were studied.

Series VI.—*Bile and strychnine injected into frogs.*

In a few experiments strychnine was mixed with the bile i tain proportions and kept for some time before injection. In of the experiments, however, bile and strychnine were inj consecutively in different parts. The doses of strychnine known from many previous experiments to produce eith effect or a very slight one; besides which facts, in many c tests the dose employed was carefully controlled.

At the outset it will be well to state that in all cases the tion of a subminimum dose of strychnine brought out perceptible effects which were not seen in the control experiments with strychnine or bile alone.

*Group I.*—Bile from nephrectomized animals and strychnine were mixed in proportion of 1 : 10,000, kept for 24 hours, and then injected into frogs.

Small frog A, control; injected into d. l. s.* 0.01 mgr. strychnine nitrate at 3.17 P.M. Observed until 5 P.M.; no increase of reflexes. Next day normal.

Frog A′ size of control; injected into d. l. s. 0.1 of a mixture of 0.1 bile + 0.01 mgr. strychnine at 3.22 P.M.; 4.07 reflexes distinctly increased; 4.15 violent tetanus. Next day again normal.

Small frog B, control; injected into d. l. s. 0.02 mgr. strychnine nitrate at 11.23 A. M. At 12.15 P.M. no increase of reflexes; 12.55 reflexes slightly increased; 1.10 on handling, a mild tetanic attack which soon passed over.

Frog B′ size of control; injected into d. l. s. 0.2 of a bile mixture of 0.2 bile + 0.02 mgr. strychnine nitrate at 11.28; 12.04 violent tetanus.

Large frog C, control; injected into d. l. s. 0.05 mgr. strychnine nitrate at 11.52 A.M. 12.53 P.M. no increase of reflexes; 1.15 reflexes increased; 2.10 reflexes markedly increased; 2.22, violent tetanus.

Frog C′ size of control; injected into d. l. s. 0.5 of a bile mixture of 0.5 bile + 0.05 mgr. strychnine nitrate at 12 M. 12.40 P.M. marked increase of reflexes; 12.53 violent tetanus.

The presence of a tetanic element in the bile in this group of experiments is quite evident. The doses of bile employed never alone produced hyperæsthesia, to say nothing of tetanus. Nevertheless one sees in the three experiments a marked increase in the efficiency of the strychnine produced by the addition of the bile.

*Group II.* Frog A, control; injection of 0.01 mgr. strychnine nitrate. No effect. Frog A′ injected 0.01 mgr. strychnine + 1.5 salt solution (0.85 %). After 3½ hours very slight temporary increase of reflexes (?). Frog A″, 0.01 strychnine + 3 c.c. salt solution. After 3½ hours slight temporary increase of reflexes (?). Frog A‴, 0.01 mgr. strychnine + 5 c.c. salt solution; same as the others. All practically normal.

Frog B, injected 1.5 c.c. bile from common duct of a normal rabbit at 10.30 A.M. Condition normal; next day the same. Frog B′, injected 1.5 c.c. same bile as above and immediately after injected 0.01 mgr. strychnine nitrate at 10.30 A.M. At 2 P.M. reflexes markedly increased, and at 2.10 on handling, tonic rigidity. At 6.30 reflexes still increased. Next day frog perfectly normal.

Frog C, injected 3 c.c. of the above bile at 10.30 A.M.; at 2 P.M. increased reflexes; soon coma; at 6 P.M. dead.

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* The abbreviation d. l. s. will be employed to designate the dorsal lymph-sac.

Frog C′, injected 3 c.c. bile and immediately after 0.01 mgr. str nitrate. At 2 P.M. increased reflexes; no coma; at 6.30 P.M. reflexes m increased and no coma. Next day animal perfectly normal.

Frog D, injected 5 c.c. of normal bile; at no time increased reflexes and paralysis soon appeared, and later in the day the animal was foun

Frog D′, injected 5 c.c. of normal bile at 11 A.M.; animal soon droo jected 0.01 mgr. strychnine. One hour after the injection of stryc tetanus broke out; at 6 P.M. animal still tetanic. Recovered next day mained perfectly normal.

This group of experiments is very instructive. Do strychnine, the ineffectiveness of which was established in controls, brought out distinct specific symptoms when with bile, and with the increase of the dose of bile, althou dose of strychnine remained the same, the strychnine sym increased in intensity, so as to bring on a distinct tetanic lasting many hours. This result can only mean that norm contains a tetanic element or a substance which in its ef similar to that of strychnine.

Moreover, this experiment demonstrates a still more imp fact. In C and D doses of three and five c.c. of norm produced coma, paralysis, and death, whereas in C′ and the addition of a subminimum dose of strychnine not onl a tetanus brought out, but coma and paralysis were als vented and the animals survived the fatal dose of bile.

It will be recalled that the bile from the common du certain rabbits produced, in comparatively large doses, æsthesia and convulsions. This result means that in these the tetanic element is present in at least minimum toxic do

The bile of some other rabbits causes in comparatively doses coma and paralysis. We have demonstrated that this bile contains the tetanic element. That this elemen to manifest its presence when injected without the addit strychnine is probably due to the overpowering influence depressing element of this bile. Some of the cited experi show, and it will be shown more fully later, that the depr substance in bile, when present in effective strength, is c completely of effacing the effects of a tetanic element strychnine or a substance of metabolic origin.

It will be recalled also that the bile of some rabbits could be injected into frogs even in quantities of three or four c.c. without toxic effect of any kind. The explanation of this fact may be that in these specimens none of the toxic agents was present in such strength in the doses mentioned as to have a toxic influence. We have, however, reason to assume that in at least some instances the tetanic elements were strong enough in the doses employed to produce hyperæsthesia and convulsions. The harmlessness of this bile may, however, have been due to the simultaneous presence of the depressing element in quantities sufficient to neutralize the tetanic effects. It has been shown above that in some cases the dose of the bile alone would produce coma, but when a subminimum dose of strychnine was added to it the effect might be a tetanus. Hence the tetanic element of the bile plus the subminimum dose of strychnine were capable of antagonizing to such a degree a fatal dose of the depressing element as to produce a toxic effect in the opposite direction. We can under these circumstances easily conceive that were the strength of the tetanic element in the bile slightly less, the result would be a simple neutralization, and neither coma and paralysis nor tetanus be developed but the frogs would have appeared simply normal.

These neutralizations might occur in many ways. There might be, for instance, a certain kind of bile which in a certain dose contains exactly a minimum dose of the depressing element; the result would be coma and paralysis were it not for the simultaneous presence of the antagonistic tetanic element in a strength weak but sufficient to neutralize a fraction of the depressing element, thus reducing it to an ineffective strength. Or the tetanic element might be present in an exactly minimum strength and the depressing element in a fraction of a miminum dose. Neutralizations might perhaps also occur when both antagonistic elements are present in minimum doses or above them, provided they are present in such proportions as to be exactly sufficient to neutralize one another. As a rule, however, as will be seen later, such mixtures as the last do not lead to neutralization.

It should be stated that the cases in which the paralyzing of three and five c.c. of normal bile was completely ove and turned into the opposite effect by the addition of s nine, were not of frequent occurrence. We met them n in bile in which the depressing effect came out only administered in still larger doses. As a rule, when mo doses of bile were sufficient to produce complete paralysi coma, the addition of strychnine was of no avail or had ra contrary effect, as we shall see later. In cases, for ins where the bile produced complete coma and paralysis v dose of two c.c., we never succeeded in overcoming pletely the effect by the addition of strychnine, although in these instances some degree of neutralization was at noticed. The following experiment illustrates this effect:

*Group III.*—Bile from the dilated common duct of a ligated three days before.

Frog A, injected 0.6 c.c. bile; complete coma and paralysis in on

Frog A′, injected 0.6 c.c. bile + 0.005 mgr. strychnine nitrate; some increased reflexes, condition decidedly better than A.

Frog B, injected 1 c.c. bile; after one hour coma and paralysis, bu

Frog B′, injected 1 c.c. bile + 0.005 mgr. strychnine nitrate; coma ar alysis very soon; dead at the end of an hour.

Here in A′ the addition of a very small dose of strychnin apparently instrumental in reducing the toxic effect o depressing bile. (The effect in B′ will be discussed later.)

The last two series of experiments have brought out two important facts: 1. That rabbit's bile surely contains a te element. 2. That the depressing and the tetanic eler within the bile are antagonistic to one another and are ca at least to a degree of mutual neutralization. Regardin mutual relations of the antagonistic factors as they act exist within the bile we have so far learned the following tl

In the bile of the gall bladder and the ligated common the depressing element is apparently the chief factor, th producing only coma and paralysis; it is toxic in comparat small doses. The presence of the tetanic element can be det only by the addition of an ineffective dose of strychnine an not in a very pronounced manner. Perfect neutralization is impossible.

The bile obtained from a biliary fistula of the common duct in general is far less toxic than that from the gall bladder. Rabbits show individual variations. The bile in some is already toxic in the comparatively moderate doses of two c.c. and less. The depressing element is predominant, the effects produced being coma and paralysis. This bile it is impossible to neutralize by the addition of strychnine. However, the doses which are not yet toxic permit without difficulty the bringing out of the tetanic effects by the addition of an otherwise ineffective dose of strychnine.

In still other animals the depressing element seems to be absent, at least it was so in doses up to ten c.c. This bile alone will produce a tetanus readily but only when given in the large doses of five c.c. or more. The addition of an ineffective dose of strychnine will bring out a tetanus with a somewhat smaller dose, but not with doses smaller than two c.c. In this bile, apparently, the tetanic element is also present in small quantities.

Finally, the bile of some rabbits produces hyperæsthesia in doses of two or three c.c. and coma and paralysis in larger doses. The addition of strychnine to comparatively small doses of this bile (one to three c.c.) will produce a tetanus. The addition of strychnine will bring out a tetanus also with doses of this bile just sufficient to bring out (without strychnine) coma and paralysis. When the dose of bile is distinctly above this minimum, coma and paralysis will be the result even if strychnine is added. Both elements are present and struggling against each other. In larger doses the depressing element is the victor, in smaller doses the tetanic element is capable of manifesting its presence, but produces only moderate symptoms. In doses near the border line the addition of strychnine is capable of turning the balance in favor of the tetanic element.

These various observations seem to demonstrate the following:

1. That for the depressing element there is a definitely effective minimum which, when present in a dose of any bile, overcomes

nearly completely the antagonistic tetanic element, no n
in what strength the latter is present.

2. That bordering on this effective minimum there is an
minimum strength of the depressing element which,
present alone, or nearly so, can produce coma and para
but this effect can be overcome by the antagonistic tetani
ment when simultaneously present in the bile in a str
sufficiently above its minimum.

3. That this last minimum of the depressing element
strength weaker but near to it, when mixed with a mini
effective dose of the tetanic element might lead through m
neutralizations to normal effects.

4. That the tetanic element when alone is efficient in a
smaller strength than the strength which is required for th
pressing element to be efficient; in other words: the effe
minimum for the depressing element lies higher than that fo
tetanic element.

With these relations of the antagonistic elements in min
can easily understand why some bile causes only depres
other bile only hyperæsthesia and convulsions, and still
bile produces hyperæsthesia in smaller and coma and par
in larger doses.

Remembering these considerations it may be further
ceived that bile which would cause hyperæsthesia or
tetanus in small doses, might produce normal conditions
larger doses, and coma and paralysis with still larger doses.
apparently paradoxical conditions would occur when
antagonistic elements were present in the bile simply in relat
minute strengths. The gradual increase of the dose of the
would first bring the tetanic element up to its effective mini
while the depressing element would still be far below its effe
strength, and the result would be hyperæsthesia or convul
A further increase in the dose of the bile would bring th
pressing element up to a strength approaching its effe
minimum, or just sufficient to neutralize the tetanic ele
even in its present strength; the result would be an appar
normal condition. Finally, a further increase of the do

bile would bring the depressing element up to the effective strength which is capable of completely overcoming the tetanic element in any strength; hence the coma and paralysis.

These paradoxical effects have actually been present in a few instances, especially in the experiments with additions of subminimum doses of strychnine. The following protocols are illustrations.

*Group IV.* Frog 1, injected 1.5 c.c. of bile from nephrectomized animals at 11.20 A.M. 2 P.M., frog normal. 6 P.M., rigidity of legs on handling; next day normal.

Frog 2, injected 3 c.c. of same bile at 11.10 A.M. 2 P.M., normal. 2.30 P.M., somewhat "stupid," otherwise normal, no increase of reflexes; next day normal.

Frog 3, injected 5 c.c. of same bile at 11 A.M. 2 P.M., coma and paralysis. 6.30 P.M., dead.

In this experiment Frog 2 had no rigidity or increased reflexes and was in better shape than Frog 1, while Frog 3 had coma and paralysis.

*Group V.* Frog 1, injected 1.5 c.c. bile from nephrectomized rabbit and immediately after injected 0.01 mgr. strychnine at 11.20 A.M. 2 P.M., violent tetanus, passed into coma. 6.30 P.M., found dead.

Frog 2, injected 3 c.c. of same bile, then 0.01 mgr. strychnine at 11.30 A.M. 2 P.M., increased reflexes. 6.30 P.M., increased reflexes, somewhat comatose (?). Next day normal.

Frog 3, injected 5 c.c. of same bile, then 0.01 mgr. strychnine at 11 A.M. 2 P.M., coma and paralysis. 6 P.M., dead.

Frog 2 with the larger dose of bile had neither tetanus nor coma and survived, while Frog 1 with the smaller dose showed both and died. The rapid development of coma after a violent tetanus we will discuss later.

*Group VI.* Frog A, injected normal bile 2 c.c. at 10.30 A.M. At 2 P.M., reflexes increased. 6 P.M., normal.

Frog A′, injected normal bile 2 c.c., then 0.01 mgr. strychnine at 10.30 A.M. At 2 P.M., marked increase of reflexes, rigidity on handling; next day normal.

Frog B, injected normal bile 3 c.c. at 10.30 A.M., at 2 P.M., stupor and paresis; at 6 P.M., coma and paralysis; next day dead.

Frog B′, injected normal bile 3 c.c., then 0.01 mgr. strychnine at 10.30 A.M. 2 P.M., normal; 6 P.M., normal; remains normal next day.

Frog C, injected normal bile 5 c.c at 11 A.M.; 2 P.M., coma and paralysis; 6 P.M., dead.

Frog C′ injected normal bile 5 c.c., then injected 0.01 mgr. strychnine at 11 A.M.; 2 P.M., coma and paralysis; 6 P.M., dead.

In this experiment the addition of strychnine to two centimeters of bile brought rigidity, while the control a remained normal; and the addition of the same amou strychnine to three cubic centimeters of bile brought normal conditions while the control died from coma and par Finally, the addition of the same amount of strychnine t cubic centimeters of bile did not reduce the paralyzing effec large dose of bile, and there was practically no difference bet the frog given strychnine and the control. The additi strychnine brought out then first tetanic symptoms; whe quantity of bile and with it the strength of the depressing ele were increased, the antagonistic elements were just sufficie neutralize one another. When, however, the depressing ele was increased considerably, it not only neutralized all te effects but had a sufficient balance left to produce paralysi coma.

These striking results were, however, rarely met with. would seem to depend upon so many circumstances more c uncontrollable as to make their intentional or even accid production infrequent. But instances of mutual neutraliz of less striking character were much more frequently produ

The addition of ineffective doses of strychnine to the b nephrectomized rabbits brought out instructive observa From the results in Series IV, we had reasons to surmise t certain amount of a tetanic element of metabolic origin, wh normally eliminated through the urine, passed in nephrecto rabbits through the bile. We compared in a number of e ments bile from nephrectomized rabbits with that of n animals as to the effects of the addition of strychnine. I majority of experiments the difference was very striking favor of the bile from nephrectomized rabbits being ren more tetanic than normal bile by this addition. Some c experiments follow:

*Group VII.* Frog A, injected at 3 P.M. 3 c.c. of normal bile, and t 3.25 P.M. 0.01 mgr. strychnine. No signs of tetanic effect; paralysis next day.

Frog A′, injected at 2.30 P.M. 3 c.c. bile from nephrectomized rabbit, 3.20 0.01 mgr. strychnine. At 3.40 legs rigid, later tetanus on handling; normal next day.

Frog B, injected 5 c.c. normal bile at 2.40 P.M, and at 3.25 added 0.01 mgr. strychnine. No signs of a tetanic influence. Coma, paralysis, and death.

Frog B′, injected 5 c.c. of bile from nephrectomized rabbit at 2.40 and at 3.25 added 0.01 mgr. strychnine. At 4.25, distinct rigidity on handling, and at 5, distinct tetanic attacks. Normal next day.

*Group VIII.* Frog A, injected at 12.30 P.M. 5 c.c. normal bile, and immediately after 0.01 mgr. strychnine; 2.30, coma and paralysis; dead next day.

Frog A′, injected at 12.30 P.M. 5 c.c. of bile from nephrectomized rabbit, and immediately after 0.01 mgr. strychnine. 2.30, increased reflexes and rigidity of extremities; no coma; next day reflexes still increased, and on hitting table, mild tetanus. Survived.

In these and in some additional experiments it was shown that the addition of ineffective doses of strychnine to comparatively large doses of bile of nephrectomized rabbits produced tetanic symptoms, while the same doses of strychnine and normal bile produced coma and paralysis. In other words, nephrectomy apparently increased the tetanic action of the bile.

We have up to now dwelt chiefly on our observations upon the increase of the tetanizing effects of bile by the addition of ineffective doses of strychnine. We have now to state that under certain conditions the addition of strychnine favors the appearance of the depressing effect of bile. Indeed among the experiments described above were some in which this action of strychnine in favoring the appearance of coma and hastening the death of the frogs was shown. This effect has been noted by us in the case of the bile from a large number of rabbits as well as other animals. These observations may be summed up as follows: When in employing doses of bile which, when injected alone, never produced any degree of coma and paralysis, there was added strychnine, no matter whether in small or large doses, it never favored the appearance of the depressing phenomena; if, however, the bile caused alone the appearance of coma and paralysis quite readily, and the dose employed was one producing sooner or later some degree of coma and paralysis, then the additional injection of strychnine frequently hastened the onset and increased the rapidity of development of the depressing effects In some cases the sudden onset of coma was preceded by very brief and rarely by very violent tetanus. The tetanus was usually so brief that it may in other instances have frequently been overlooked. The larger the added amount of strychnine, the more quickly the tetanus and the sooner complete coma and paralysis would set in.

In cases where coma and paralysis are brought on by bile alone, the development of the depressing symptoms takes place gradually and slowly, a fact especially true of doses not too large. The usual course is as follows: After the animal recovers its activity immediately after receiving bile it gradually becomes abnormally quiet and remains sitting in one place with the head gradually sinking down to the table. At this stage handling arouses it for a while and it jumps away in a normal manner, but as soon as left alone it becomes again droopy. Soon it offers little resistance, very often it does not jump away, and when it does jump it does so clumsily for short distances. When turned on its back it makes only feeble attempts to turn on its stomach. However, once in a while it succeeds, and when again on its stomach draws its legs near the body, the head and stomach now resting on the table. Gradually the limbs become limp and in the attempts to move they get away from the body; their tonicity is lost and they look œdematous, after which the animal soon appears to be perfectly paralyzed. Occasional sudden, feeble twitchings of some parts of the body occur, the respiration becomes feebler and slower, the heart-beats become diminished. At this stage the pupils are generally constricted. The frog can remain in this condition for many hours before all signs of life disappear. Between the injection and the state of complete coma and paralysis many hours often elapse.

The course is entirely different when in addition to an effective dose of bile a dose of strychnine, especially one approaching the effective minimum, is given. On being put under the glass bell the animal becomes very lively, keeps its head high, and is continually moving around; it is always near the glass wall looking for opportunities to escape. On hitting the table suddenly or handling the animal, and sometimes even without any visible cause, the frog is seized with a tetanic convulsion and a few seconds later is lying stretched out as nearly paralyzed as a frog in advanced stage of depression from bile alone. A blow on the table may still bring on brief responses of feeble stretching of the extremities, but in a short time there is no visible difference between the paralysis of the strychnine frog and the paralysis brought on simply by the bile.

As to the cause of this paradoxical effect of strychnine, there are several possible explanations. We may think in the first place of the possibility that strychnine, containing as is well-known a certain depressing element, manifests this action after the tetanus is over, and hence we might assume that it is this depressing effect added to that of the bile which hastens the onset of complete depression. It is probable also that the sudden onset of coma is always introduced, as we have already mentioned above, by a brief tetanus which on account of its slightness is frequently overlooked. We may then assume further that after the convulsive explosion, the resistance of the tetanic element becoming momentarily exhausted, the depressing phenomena appear suddenly. Finally, the normal interval between the injection of the bile plus the strychnine and the outbreak of acute coma is upheld by a very unstable equilibrium between the two antagonistic elements. The sudden outbreak of the tetanic condition disturbs this equilibrium, with the result that henceforth the stronger factor, in this instance the depressing element, dominates the field. It is perhaps possible that the acute outbreak is brought about by a combination of all the factors mentioned in the three possibilities.

We have stated that after the outbreak of the coma and paralysis the frogs remained in a state of depression until death. This is the usual outcome. In a few instances, however, the course up to the acute onset of the coma was the same as in all the other experiments. The further part of the course, however, was strikingly different. The animal would lie apparently in complete coma and paralysis; but a stimulus of any kind—such as touching the frog or striking the table—would bring on a pronounced tetanic attack of short duration which would pass rapidly into complete coma and paralysis. Sometimes these tetanic attacks would break out apparently spontaneously. alternation between tetanus on the one hand, and coma paralysis on the other, would continue for many hours, the co tion reminding one in a striking way of the syndrome in hu pathology designated "eclampsia."

The quantity of strychnine employed in these cases had t at least near the minimum effective dose; the quantity of on the other hand, had to be not too much above the minin dose which when employed alone brought on coma and paral When large doses of depressing bile were injected no such e could be brought on by the addition of even a large dos strychnine.

The tetanus occurring in these "eclamptic" frogs was mark in contrast with the tetanus brought on by bile alone. E single tetanic attack in the latter cases lasted a long time, after passing off left behind a marked tonicity of the mus giving to the animal a somewhat "graceful" appearance. I tetanus in the "eclamptic" state was very brief and pa abruptly over into perfect relaxation, which gave the animal "œdematous" appearance.

Finally, we have to add that, when after the injection of coma and paralysis were already completely developed, injection of strychnine even in doses of 0.04 or 0.05 milligran hardly ever exerted any influence.

The paralysis which bile produces is in the first place of cen origin. This fact has been demonstrated by Rywosch others by direct experiment. We repeated one of the exp ments. In a completely paralyzed animal which received the injection, electrical stimulation of the cord produced no ef upon the muscles of the trunk or the extremities; while stim tion of the sciatic nerve produced quite active contractions of extremities.

That bile also affects nerve and muscle directly was sh long ago by many observers. In our experiments we freque had occasion to observe this effect in the following man In order that the large quantities of bile which we introdu should not escape again, we injected the lymph-sacs by pas the needle through the muscles of the thigh, the muscles thus working as a valve. Now these legs, and especially the thigh, were often paralyzed long before there was any sign of paralysis in the rest of the frog. In some instances the frog would respond with a tetanus in which even the lower part of the paralyzed extremity would take part. The paralysis of the thigh in those cases was caused undoubtedly by the bile coming in direct contact with the muscles of the thigh. This would mean that even that bile which failed to produce paralysis through the central nervous system might still reduce the irritability of the muscles by coming in direct contact with them.

The reduction of the irritability and conductivity of nerves and muscles by bile had probably some share in the advanced stages, and possibly also in the earlier stages, of paralysis. Of the latter we had to think especially when the injection of bile was given into the lymph-sacs, while the animal remained in its normal position, as was the rule. It might thus happen that some bile reached the sciatic plexus or nerves and caused a premature local paralysis of the posterior extremities. Indeed we met with these examples in cases in which ineffective doses of strychnine in addition to bile were employed, and in which the front legs became rigid while the posterior extremities were paretic or paralytic. In a few cases, therefore, immediately after injecting the bile into the lymph-sacs, we have suspended the frogs by the legs, causing the bile to accumulate preferably in the anterior parts of the body. In these experiments it seemed, indeed, that the addition of strychnine brought on rigidity of the posterior extremities very readily and distinctly, while the animal seemed otherwise to be in a state of stupor.

We should mention further that of the rabbits' bile which we have employed some was received sterile, some was sterilized, and some was exposed to boiling for half an hour before it was used. A few of the experiments with the last bile made the impression that boiling reduces the activity of the depressing element and favors the appearance of tetanus. However, the analysis of all the experiments does not positively justify such a claim. It may be stated with safety that boiling does not affect in a pronounced manner either of the biliary elements descri But in a number of comparative experiments it was shown t boiling reduces the toxicity of the bile as a whole. The anin which received boiled bile survived pronounced attacks of teta as well as some conditions of coma and paralysis, whereas all control animals with the same amount of unboiled bile, wh otherwise produced the same symptoms, died a day or two a the injections. Our experiments were not extensive enough this regard to permit a more definite statement.

The numerous facts communicated in the foregoing pages w derived, as has frequently been stated, from the study of rabb bile. We also stated that the toxicity of the bile of other anim and of bile salts was studied. These latter studies were not full as the other and were restricted to ascertaining a few essen facts. The results of these studies will now be reported briefl

*Guinea-Pigs' Bile.*—The bile of only three guinea-pigs obtai from a cannula fastened either in the common bile duct or in gall bladder was at hand. The quantity yielded was small our observations therefore are limited to twelve frogs.

Two or even three c.c. of fistula bile caused no toxic effects of any kind. c.c. of stagnated bile from the gall bladder also had no effect. The additic 0.01 mgr. strychnine did not bring on hyperæsthesia. The addition of mgr. strychnine brought on, after many hours, distinct rigidity, while control animal remained normal.

These few results are recorded without attaching much portance to them. The observations were too few and too li varied to permit positive conclusions. It may be said, howe that, few as they were, they were more numerous than some fr which certain writers drew their conclusions regarding the nat of the toxicity of bile.

From the fact that even three cubic centimeters were with toxic effect we were at first inclined to the general conclusion t guinea-pigs' bile is less toxic than rabbits', but this notion dissipated by the experiments with rabbits' bile in which toxic dose was definitely greater than this amount. It will t a more extensive study to decide whether guinea-pig's bil indeed less toxic than rabbit's.

*Dogs' Bile.*—Our experience with dogs' bile is also limited. The few experiments made, gave, however, some definite results in accord with those obtained with rabbits' bile.

*Group IX.* Frog 1, control, injected 0.01 mgr. strychnine at 3.50 P.M. No effect.

Frog 2, injected 0.5 c.c. boiled dogs' bile (from fistula) at 3.50 P.M. No effect.

Frog 3, injected 0.5 c.c. boiled dogs' bile followed by an injection of 0.01 mgr. strychnine at 3.50 P.M. At 4.30, exaggerated reflexes followed soon by typical tetanus.

In this experiment 0.5 of the bile had no effect, but the addition of an ineffective dose of strychnine brought out a typical tetanus. That means that 0.5 of the dogs' bile contained a tetanic element large enough to convert a subminimum dose of strychnine into a toxic one.

*Group X.* Frog 1, weight, 36 grammes; injected 1 c.c. of dog's bile at 10.15 A.M. At 12 M., complete coma and paralysis.

Frog 2, weight 42 grammes; injected 1 c.c. of dogs' bile and immediately afterwards 0.01 mgr. strychnine at 10.15 A.M. At 10.50, complete coma and paresis. 12 M., dead.

One c.c. of dogs' bile caused complete coma and paralysis in about two hours; the addition of strychnine hastened the onset of the depressive phenomena and death, an experience similar to that frequently seen in case of the rabbits as described.

Hyperæsthesia and convulsions did not occur after injections of dogs' bile without the addition of strychnine. Still our experience with this bile was too small to permit the drawing of any conclusion.

*Ox Bile.*—Ox bile as it was obtained from the slaughter-house and inspissated bile dissolved in various concentrations were employed. The bile frequently was filtered and sterilized before using. Our experience with this bile was quite extensive, and hence the few results reported were conclusively established. It is desirable to keep in mind when comparing the effects of ox and rabbit bile that all of the former was obtained from the gall bladder.

Ox bile is very toxic; 0.3 c.c. will, as a rule, cause sooner or later toxic symptoms and death. The former are nearly exclusively depressive, namely: coma and paralysis. In our experiments we came across tetanus once only. This f received 0.3 c.c. of bile; first developed coma; but the next the coma was interrupted by tetanic attacks—in other wo an eclamptic state supervened. Smaller doses never gave e hyperæsthesia. Since this effect is similar to that obtained w rabbits' bile taken from the gall bladder or from the dila common duct, stagnated bile is apparently very toxic, depressing element being the controlling factor in it. We served, however, numerous proofs of the existence of a teta element even in this depressing bile.

*Group XI.* Frog A, received 1 c.c. of a 10 per cent. solution of ox b Condition good; no effect.

Frog A', received 1 c.c. of a 10 per cent. ox bile followed by 0.005 mgr. stry nine at 4 P.M. At 6 P.M., increased reflexes.

Frog B, received 1.25 c.c. 10 per cent. ox bile; condition remained good.

Frog B', received 1.25 c.c. 10 per cent. ox bile followed by 0.005 mgr. stry nine at 4 P.M. At 5 P.M., violent tetanus.

In this series neither the doses of bile employed alone strychnine alone produced any effect. When, however, bo were given, the effects produced were hyperæsthesia and som times tetanus.

Moreover, in some instances when the toxic dose of bile w on the border line and tended sooner or later to bring on pressing symptoms followed by death, the addition of strychni still brought out tetanus. Of this class of effects the next expe ments form examples.

*Group XII.* Frog 1, injected 0.25 c.c. ox bile at 11.30 A.M. At 3 P marked stupor. Next day, dead.

Frog 2, 0.25 c.c. ox bile, followed by 0.01 mgr. strychnine injected at 11 A.M. At 3 P.M., slight stupor. Next day, distinct tetanus.*

On the other hand, we observed many instances in which a do of 0.01 milligramme strychnine or more, when superadded to distinctly toxic dose of ox bile, hastened the onset of the depr sive symptoms and death. In some of these the coma, which was brought on acutely by the addition of strychnine, was not of a continuous, but rather of an "eclamptic" character.

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* Such effects remind one of the action of certain doses of morphine u frogs. On the day of injection there is either no effect or a moderate stup while a day or two later a distinctly tetanic condition develops.

There were some slight variations in the toxicity of the bile obtained at different times. Whether this variation was due to individual differences in the animals we cannot state, since we are not sure that each portion of bile was obtained from a single animal.

Ox bile is therefore highly toxic in small doses and exceeds in toxicity gall bladder bile of rabbits; but like the latter its effect is essentially depressive. The addition of ineffective doses of strychnine shows, however, that ox bile contains an effective tetanic element, the manifestation of which is suppressed by the superior antagonistic effect of the depressing agents. In other words, by the addition of strychnine to ox bile all the phenomena observed in the study of bile from the common duct of rabbits plus strychnine were produced.

*Bile Salts.*—Glycocholate and taurocholate of sodium were studied separately. According to Rywosch,[19] the fatal dose of sodium taurocholate for frogs weighing from 40 to 50 grammes is about 60 to 70 milligrammes, and of sodium glycocholate about 100 milligrammes. Judging by his protocols Rywosch had in mind doses which produced coma and complete paralysis in less than half an hour and death in less than two hours. In most instances the frogs which we used weighed between 30 and 35 grammes. We considered the bile salts toxic when a given dose produced pronounced toxic symptoms within a few hours. These experiments may be summed up briefly as follows:

A few frogs responded to injections of 20 milligrammes of bile salts, and more responded with distinctly toxic symptoms and a fatal result on the day of injection, or within the next few days to 30 milligrammes. In some instances even 40 milligrammes were without positive effect, and exceptionally some effect was obtained with 15 milligrammes. Fifty milligrammes or more were invariably highly toxic.

The toxic symptoms were stupor and complete coma, paresis, and complete paralysis. Beginning with small quantities and gradually increasing the doses of the bile salts until definite and fatal symptoms set in, we came across only one instance for each salt of a tetanic effect of doubtful character. The exceptions were as follows: With a dose of sodium glycocholate of 40 milligrammes, there were no perceptible symptoms on the same day, but on the next day some tonic rigidity seemed to be present. In another instance about half an hour after injection of 25 milligrammes of sodium taurocholate coma developed, and while handling the frog a slight rigidity set in which soon passed over into complete paralysis.

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[19] Rywosch, l. c.

These two doubtful instances of temporary rigidity were the only signs of the presence of a tetanizing influence among the experiments with the bile salts. There was in this regard no perceptible difference between the effects of sodium taurocholate and glycocholate. It should also be added that in our experiments no difference in the toxicity of the two salts was noticed, a point of difference with Rywosch.

The experiments with the addition of strychnine did not bring out any striking results which favor the notion of the presence of a tetanic element in the salts. Less than 0.01 milligramme had not the slightest effect. Of many experiments with additions of 0.01 milligramme of strychnine to the dose of sodium glycocholate, in one only was slight hyperæsthesia noted. This amount of strychnine when added to sodium taurocholate (10 mgr. salt + 0.01 mgr. strychnine, mixed) produced in one case, after 2½ hours, a passing tetanus, and in another case (30 mgr. + 0.01 mgr.) a slight tetanus. In the many other experiments with the taurocholate (strychnine 0.01 and 0.015) there was no effect except perhaps a slight hyperæsthesia in one or two instances. The addition of 0.02 milligramme of strychnine to sodium glycocholate brought out tetanus in two experiments, while the control with strychnine alone gave only hyperæsthesia; in other instances there were only increased reflexes. We had the same experience with sodium taurocholate.

In a few instances the addition of a larger dose of strychnine hastened the onset of coma. Two of the above-mentioned attacks of tetanus were the precursors of coma and paralysis which continued until death, except in two examples where the course was of a typically "eclamptic" character.

The general conclusions which our experiments with bile salts permit us to draw are that the toxic effects of the salts upon the general nervous system are essentially depressing in character. The salts probably also contain the tetanic element, but in very small amount, hence the addition of strychnine is distinctly less effective than even with bile which comes from the gall bladder.

In reviewing our results we shall trace our steps backwards. The investigation, which we began in the attempt to solve our original problem, led us to the question of the actual nature of the toxic action of bile. As an answer to this question we obtained a number of facts the signification of which we wish to present.

It is now generally accepted that the toxic effects of bile are only of a depressing character. In our studies we found this to be true for the bile obtained from the gall bladder of the rabbit and ox, at least as far as the actual effect is concerned. In a few instances, in which we made experiments, this fact held good for the gall-bladder bile of dogs and guinea-pigs. Sodium taurocholate and glycocholate also proved to have a depressive action.

It was, however, different with bile obtained from a fistula of the common duct of rabbits. The bile of certain rabbits produced hyperæsthesia and true tetanus, but never coma and paralysis. The bile of other rabbits produced marked hyperæsthesia with small doses, and coma and paralysis with large ones. Only in a few experiments did this bile cause coma and paralysis and no increase of excitability. Hence we have positive and direct proof that normal bile from many rabbits possesses an exciting element capable of producing clonic and tonic convulsions in frogs.

We found that small doses of bile of any kind could cause hyperæsthesia and tetanus when supported by small doses of strychnine. The doses of the bile and strychnine which come into play in these experiments were below that which separately produced symptoms. This could only mean that bile contains an element which like strychnine can produce increased excitability, and which can supplement it. We designate this hypothetical agent in bile for sake of brevity as the tetanic element. By the same method we have demonstrated the existence of a tetanic element in the bile of the common duct of rabbits which alone gave only depressing symptoms, and in the gall-bladder bile of dog, ox, and rabbit. The evidence obtained for the presence of the tetanic element in bile salts was not conclusive. If present at all, it apparently occurs in smaller quantity than in the whole bile.

We may, therefore, claim from our experiments that we have established the fact that bile from the common duct of some animals, at least, contains a tetanic element which may manifest its presence without the aid of strychnine; and, in some instances, would appear to be the sole toxic agent present.

Gall-bladder and stagnant bile of all animals produce depressing symptoms, although they surely contain a tetanic element.

From Series II and III it would appear that bile which is obtained soon after the preparation of a fistula is apt to cause depressive symptoms chiefly.

In some animals indeed all the bile from the common duct causes in comparatively small doses only coma and paralysis. It should be added that the flow of bile in these animals was slow, and the quantity collected was small as compared with that obtained from animals the bile of which produced hyperæsthesia and convulsions. All these facts considered, it may be stated that stagnant bile produces depressing symptoms.

Our experiments have demonstrated not only that all kinds of bile contain the tetanic element, but also that this and the depressing element are present simultaneously. In stagnant bile, though it surely contains both elements, it is, apparently, the depressing element essentially which manifests its effects. What are the mutual relations of these two toxic agents? From our experiments with the addition of strychnine we may answer this very difficult question as follows:

1. That the depressing effect of large doses of bile cannot be reduced or visibly modified by the addition of any dose of strychnine.

2. That the depressing effect of doses of bile, in quantities near the toxic minimum, can be promptly modified by a subminimum dose of strychnine: (a) The effect can be neutralized or annihilated, whence a normal condition results; (b) the effect can be overcome to such an extent as to bring out the opposite or tetanic effect.

3. That by an increase of the depressing element the positively tetanic effect of strychnine plus the tetanic element in the bile can (a) be neutralized, i. e., reduced to normal, or (b) made to produce coma and paralysis.

These observations lead us to assume in the first place:

*That the depressing and tetanic elements are exact antagonists and that their joint effect is that of an algebraic sum.*

That, furthermore, when each of the antagonists is simultaneously present in the bile in a distinctly effective minimum, the effect of the depressing element is by far the stronger, so as to conceal any modifying effects of its antagonist.

That the tetanic element attains its minimum effectiveness in a much smaller dose than is required for the effective minimum of the depressing element.

On the basis of these interrelations we can explain the following combinations:

When the depressing element is present in a distinctly effective minimum, the result of an intoxication will always be coma and paralysis.

When the tetanic element is present in a strength which is its minimum or above it, while the depressing element is far beneath its own minimum, the result will be hyperæsthesia and possibly tetanus.

When both elements are present in minute quantities, very small doses of the bile will be harmless. With an increase of dose a stage will first be reached in which the tetanic element will attain its minimum, but reduced somewhat in effect by a low strength of the depressing element, a condition leading to hyperæsthesia. The next increment of increase of dose will be influenced more by the depressing than by the tetanic element, through which the result may be the production of a normal condition, due to exact mutual neutralization. Any further increase of dose will, of course, lead to supremacy of the depressing element. The middle stage, which depends largely upon exact neutralization, will frequently be missed or overlooked.

The assumption that the minimum for the tetanic element lies much lower than for the depressing element,[20] and the fact that the definite minimum of the depressing element always overpowers the tetanic element, readily explain why in stagnant bile the depressing effect is supreme.

Let it be assumed for the sake of illustration that D (depressive) = 2 T (tetanic), and let it be further assumed that 4 c.c. contains D + 2 T, then 4 c.c. will give coma and 2 c.c. (½ D + T) will give tetanus. When the stagnant bile loses half of its water without losing its toxic elements, then 2 c.c. of this bile will contain D + 2 T, with the result that now 2 c.c. of this bile will cause coma and paralysis instead of tetanus.

In all of these considerations we have however to remember that in the algebraic sum of both antagonists, no matter how small one of the factors may be, none of the effect is actually lost, i. e., if the result is coma and paralysis in some degree, we have to think were it not for the simultaneous presence of the tetanic element the degree of depression would have been higher; and vice versa.

The conditions and relations which we have just described hold good for stable relations only, i. e., when both antagonistic elements exist in such firm relations to one another as to produce a state of equilibrium. When these mutual relations are disturbed, as would for instance happen when one or the other element was suddenly superadded in a certain strength, the outcome would be a struggle for supremacy, which might continue or be early decided for the one side or the other, whence the equilibrium would be broken. We have seen this happen in our experiments where the addition of an effective dose of strychnine to an effective dose of bile brought out a very short tetanic effect, which immediately gave way to a premature onset of deep coma and paralysis. The depressive phenomena were either continuous till premature death, or an alternating change between coma and convulsion, or a state of "eclampsia" occurred.

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[20] An analogy is found in the minimum toxic dose of strychnine (tetanic) which is 10 times lower than the minimum of morphine (depressive).

Finally, we have to point out that in bile salts the tetanic element is apparently present in less amount than in the whole bile. This fact does not agree well with the now generally accepted theory that the entire toxicity of bile resides in the bile salts. An assumption that the tetanic element is intimately associated with some other portion of the bile, not yet isolated in purity, would seem more in harmony with the facts; in which case it might be thought that only a fraction of this element clings to the bile salts. It should also be recalled that in our experiments the tetanic element was not destroyed by prolonged boiling of the bile. Still we do not wish to draw final conclusions from our series of experiments with bile salts.

The results of the series of experiments which we have just analyzed demonstrated that bile from the common duct of normal animals does contain a tetanic element and produces occasionally convulsions, and they also demonstrated that the addition to this bile of a tetanic factor of any source will increase the tetanizing efficiency of that bile. We shall now examine in the light of the last experience the results which we obtained in the first few series of experiments.

In Series IV we found that the bile from the common duct of nephrectomized rabbits showed a tetanic character more frequently than the bile of normal animals in Series V, and much more so than the bile of the ante-strychnine period in Series II (nephrectomized rabbits). We can now safely interpret this observation on the basis of the assumption which we previously suggested, namely, that normally a tetanic agent of catabolic origin is being eliminated in some degree through the urine.

After nephrectomy this tetanic agent finds its way, in part or wholly, into the bile and thus increases the efficiency of the tetanic agent normally present in this fluid.

This interpretation is also in harmony with the observation that the addition of strychnine to bile from nephrectomized rabbits increases the tetanizing effect of this bile more markedly than bile of normal rabbits.

But as the bile of the ante-strychnine period in Series II is, as we stated above, more or less stagnant, the prevalence of depressive symptoms of intoxication in spite of the added tetanic element is easily understood.

The bile of the post-strychnine period of Series II appeared to be more tetanic than that of any other series. We assume that in this series some at least of the strychnine was eliminated through the bile. Hence the tetanic agents in this bile were of three kinds: the tetanic element of the bile itself, the strychnine, and the tetanic element ordinarily eliminated through the urine. Through the united effort of all the three agents, the effects of the antagonistic depressing element are considerably overcome; whence the considerable increase of the exciting effects of the bile of this series.

The same reasoning applies to the bile of at least two normal animals of Series III which received strychnine. An instructive incident is the observation in these cases that the urine of these animals had nearly no hyperæsthetic effect, while in normal animals which receive strychnine the urine, as a rule, contains so much strychnine as to produce tetanus. Our explanation of the underlying conditions is as follows: Normally strychnine is eliminated through the bile into the intestines, but only to be reabsorbed and to be gradually and finally eliminated through the urine. However, as the bile is continually being discharged outside of the body through a fistula from the common duct, there is less chance for the strychnine to get into the urine.

In the first series of nephrectomized rabbits with ligated common duct the strychnine had diminished avenues of escape from the body, whence the proportionately more frequent occurrence of tetanus in these animals than in animals which were nephrectomized and then received subminimum doses of strychnine.

Furthermore, it is possible that in some cases strychnine accumulated in the gall bladder, the biological test failing to demonstrate its presence on account of the overpowering effect of the depressive element in this stagnant bile.

## RESUMÉ.

The preceding investigation can be summarized as follows:

That, contrary to the prevailing opinion, bile contains a tetanic element, or an agent which causes increase of excitability of the nervous system.

That bile from the common duct of many rabbits causes marked hyperæsthesia and tetanic attacks when injected into frogs.

That stagnant bile (as in the gall bladder, etc.) invariably produces coma and paralysis.

That the depressive and exciting elements of the bile are mutual antagonists, the total effect of both, when simultaneously present, being that of an algebraic sum.

That the depressive element when present in a highly effective dose is by far the stronger of the two; while, on the other hand, the tetanic element becomes effective apparently in a dose far below that which constitutes the minimum for the depressive element.

That owing to these peculiar characteristics of the two antagonistic toxic elements, the mixture of both within the bile can be so adjusted as to produce any of the many possible degrees of irritability lying within the range of tetanus at one end and paralysis at the other, and including a neutral state presenting apparently a normal or tranquil condition.

That bile salts seem to contain the tetanic element in a distinctly less amount than the whole bile.

That nephrectomy increases the toxicity of bile in the direction of excitation.

That the effect of nephrectomy can be imitated by subcutaneous injections of strychnine.

That the bile of nephrectomized rabbits which received strychnine produces hyperæsthesia and convulsions more readily than any other bile.

That, finally, by injecting a toxic dose of strychnine into a frog soon after it has received a distinctly depressing dose of bile, an "eclamptic" state can in some cases be produced.

# THE CONSTITUENT OF THE BILE CAUSING PANCREATITIS AND THE EFFECT OF COLLOIDS UPON ITS ACTION.

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The origin of many cases of acute inflammation of the pancreas in a diversion of the biliary secretion from the intestine into the pancreatic duct has been established by Opie's[1] observation and experiments. His experiments did not, however, take into account the nature of the biliary constituent which excites the inflammatory and other changes, neither did they undertake to discover whether varying effects were ever produced by samples of bile of different composition. While Opie's discovery threw much light upon acute hæmorrhagic pancreatitis it did not illuminate particularly the less acute or the chronic forms of pancreatic disease. It is noteworthy that the association of cholelithiasis with pancreatic disease[2] is more frequent than the mere acute affections of the gland, and the coincidence of cholelithiasis with sclerosis of the pancreas is so common as to arouse a suspicion of a possible pathological relationship of these conditions. The experiments which form the subject of the present communication are believed to show, first, the nature of the constituent of bile which acts injuriously upon the pancreatic tissue, and, second, the mechanism by which the injurious action of this constituent is modified and controlled and hence rendered capable of bringing about widely diverse effects. The experiments made upon the dog are believed not to be without application to similar pathological conditions found in man.

The bile contains two sets of constituents of highly different chemical composition: crystalline principles, and colloids. The former consist chiefly of the biliary salts and coloring matter, the latter of nucleoalbumin. The biliary salts are known to act injuriously upon cells, for which a measure may be found in the dissolving effects upon red-blood corpuscles[3]; while no such direct cellucidal property is known either for the biliary coloring matter or the mucin.

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[1] "The Etiology of Acute Hæmorrhagic Pancreatitis." *Bull. of the Johns Hopkins Hospital*, 1901, xii, 182.

[2] Opie, *Diseases of the Pancreas*, 1903.

Bile is, however, a secretion of much complexity and hence it is possible that it contains uniformly, or at times, still other substances of less defined nature which may act injuriously upon cells. The experiments to be related would seem to make unnecessary the assumption in bile of any hypothetically injurious substance upon which depends the power to set up inflammation of the pancreas.

The first experiments were made with a sample of sodium taurocholate as representing the main bile salt of the dog's bile. The specimen was brownish in color and gave on solution a yellowish-brown fluid. The salt was dissolved in a proportion approximating the biliary salt strength of dog's bile, namely, 0.650 gram of the salt in five cubic centimeters of water or saline solution.[4] The injection was made directly into the pancreatic duct as it passed into the intestinal wall. The duct was frequently tied after the injection. The later experiments with bile salts were made with samples of the total salts prepared directly from dog's bile. The method was to treat gall-bladder bile with absolute alcohol in large excess, separating the precipitated mucin and in part the coloring matter. The alcoholic extract was evaporated to dryness on a water bath with animal charcoal. The dried mass was repeatedly extracted with absolute alcohol, and the alcoholic extract after moderate concentration was precipitated with ether. The salts were quickly separated by filtration with suction and dried over sulphuric acid. They were obtained in a white state. The muciginous residue from the first alcoholic extraction was dried and used in certain experiments in which the elements of the bile were recombined.

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[3] Rywosch, *Inaugural Dissertation*. Dorpat, 1901.

[4] Shafer, *Text-Book of Physiology*, I, p. 370.

Experiment 1. 3. 15. 05. Brown mongrel. 11 A.M., injected into main pancreatic duct 5 c.c. solution of 0.650 grm. white bile salts in water. Duct tied. Loop of intestine exposed quickly became congested. Pancreas at place of injection became immediately congested, and in 2 or 3 minutes stasis or hæmorrhage occurred. 5 P.M., dog lying in farthest corner of cage; very sick 3. 16. 05., very sick; dead, 2 P.M. Autopsy: No excess of fluid in the peritoneal cavity; serosa, except for fat necroses to be mentioned, smooth and glistening. These fat necroses are typical in appearance and are scattered in the omentum and mesentery. The pancreas, except a small fraction of the duodenal end, shows swelling, hæmorrhage, and necrosis. The hæmorrhagic areas occupy spaces in the pancreatic substance and reach an extent of 2 x 3 cm. Fat necrosis also occurs in the interstitial pancreatic tissue.

Experiment 2. 3. 23. 05. Pug. 10.30 A.M. Same injection as in previous experiment. Almost immediate extreme congestion and hæmorrhage into injected portion of the organ. Dead, 3. 25. 05., during night. Autopsy: Peritoneal cavity contains much bloody fluid; serosa covered with fine, granular exudate. Fat necroses occur in the omentum, mesentery, and fat about operation wound. Adhesions occur between the liver, pancreas, and colon, which on separation disclose a cavity the size of a small orange containing dark bloody and slightly offensive fluid. The walls, composed of pancreatic tissue, are necrotic and covered with fat necroses and hæmorrhages. In other parts the pancreas shows softened areas of varying size (reaching 3 to 4 cm.), which prove to communicate with the larger cavities. The substance of the gland contains large, unsoftened necrotic foci of pancreatic tissue and fat.

The power to set up inflammation of the pancreas of high degree is proven for the bile salts by these experiments. The fatal nature of the inflammation could be further emphasized by citing other experiments, but these should suffice. In one experiment with bile salts death occurred in eight hours, by which time the entire pancreas was swollen and diffusely infiltrated with blood. The fact should be emphasized that besides the typical picture of experimental pancreatitis in the dog the bile salts have produced in one instance (Experiment 2), in the brief space of one and a half days, a condition of hæmorrhagic and gangrenous inflammation of the gland.

The next experiments relate to the action of the alcohol-insoluble or muciginous residue, in the stating of which an account is also given of a third series of experiments, in which the two separated portions of the bile were used after recombination.

Experiment 3. 3. 15. 05. 10 A.M. Brown Skye terrier. 5 c.c. of an aqueous suspension of the alcohol-insoluble residue injected. The suspension was pale brownish-yellow in color and of muciginous consistence. Duct tied.

No discoloration of pancreas except from injection; no congestion. 5 P.M. Dog bright; walks about. No subsequent symptoms. 3. 20. 05. Chloroformed. No lesion of pancreas except, possibly, slight sclerosis from ligation of the duct. No general increase in consistence of the organ.

Experiment 4. 3. 15. 05. White and brown cur. 10.30 A.M. 5 c.c. of the bile-residue suspension of Experiment 3 plus 0.650 grm. bile salts. Duct tied. Immediate discoloration followed by stasis or hæmorrhage of the pancreas in injected area. The duodenal loop of intestine became greatly congested. 5 P.M. Dog appears bright and is up. 3. 20. 05. Dog looks well. Chloroformed. General peritoneal cavity normal in appearance. The pancreas in part is swollen and hæmorrhagic. The duodenal tail is the most normal in appearance, but the tissue immediately adjacent to this is firmer than normally and of a roughened appearance. This pale, firm tissue passes into the acutely swollen part of the gland, which, on section, shows hæmorrhagic and necrotic foci. In this process the entire mid- and splenic-portions are involved.

The absence of all inflammatory power in the alcohol-insoluble residue is brought out by these experiments, which also point to a positive property of this residue, namely, that of reducing in activity the biliary salts. Since this is a property of some importance still another set of experiments will be given.

Experiment 5. 3. 1. 05. Black mongrel. 11 A.M. Injected 5 c.c. of dissolved alcohol-insoluble residue. Duct not tied. Chloroformed, 3. 4. 05. Pancreas appears normal except at the point of separation from the intestinal wall in exposing the duct. A few small necroses in the fat at this place.

Experiment 6. 3. 1. 05 White mongrel. 12 M. 0.650 grm. sodium taurocholate (Merck's) in 5 c.c. aqueous suspension of alcohol-insoluble residue. Injected portion of pancreas became immediately congested. possibly hæmorrhagic. 3. 3. 05. 10 A.M. Dog recovered from operation; looks well. Chloroformed. Peritoneal cavity shows nothing abnormal except a small quantity of bloody fluid in the pelvis. Pancreas, except duodenal end, greatly swollen, and the serous surface covered with bloody exudate. Very few areas of fat necrosis, limited to region about pancreas. On section, the swollen pancreas permits much blood-stained serum to escape. The hæmorrhagic and œdematous areas are somewhat separated, the former being in the parenchyma, the latter in the interstitial tissue chiefly. The duodenal extremity alone has escaped.

These experiments leave no reasonable doubt of the modification by other biliary constituents of the effects upon the pancreas of bile salts. The modification is expressed by, first, the less fatal character, and, second, less acute and destructive nature of the inflammation of the gland. The latter changes, as compared with the severer fatal inflammations, do not differ so much in extent as in intensity, and while hæmorrhage and œdema commonly exist marked necrosis is not so prominent. Since, however, the natural full bile is able to cause an acutely fatal inflammation we should also expect the recombined bile to do the same unless the chemical processes have permanently altered the constituents. The next experiment bears on this subject.

Experiment 7. 3. 23. 05. Brown dog. 10.45 A.M. 5 c.c. resuspended bile residue plus 0.650 grm. bile salts injected. The suspension was thinner than previous ones; mixture kept in thermostat at 60° C. for ½ hour. 3. 24. 05. 6 P.M. Dead. Autopsy: The peritoneal cavity congested, and contains excess of fluid. The pancreas is softened, congested, and hæmorrhagic. The section of the gland shows intermingled areas of necrotic parenchyma, hæmorrhages, and fat necrosis; moderate number of peritoneal fat necroses.

It was, of course, desirable to determine the nature of this inhibitive action upon the bile salts. Two possibilities presented themselves: the first, that a directly antagonistic substance exists in the bile which tends to inhibit the salts; the second, that the restraining action is bound up with the physical properties of the medium, since the proteid constituents of the bile give a colloid quality to the mixture.

Experiment 8. 3. 22. 05. Brown mongrel. 11 A.M. 5 c.c. semi-solid mixture, at room temperature, of agar-agar and gelatine was melted and in it was dissolved 0.650 grm. bile salts. Mixture kept at 60° C. for 30 minutes; cooled to 40° C. and injected. Duct not tied. 3. 30. 05. Chloroformed. Dog has lost considerably in weight. Autopsy: General peritoneum normal. Firm adhesions about pancreas, liver, stomach, and intestine. On exposing the pancreas it appears shrunken and is firmer and more nodular than normally. Only the duodenal end retains its normal appearance. The main part of the gland is grayish-white in color and infiltrated, here and there, with blood. An area of necrosis the size of a bean and extending to the surface occurs in the mid-duodenal portion. On section, the whole gland, except a superficial sheath or armor of pancreatic tissue below the capsule, is necrotic, grayish, and soft, and gas bubbles in small number slowly disengage themselves from the cut surface. Odor not offensive. On cover slips bacteria are not numerous, but of varied morphology.

That a bland colloidal solution can restrain the activity of the otherwise very highly destructive and certainly fatal bile salts is shown conclusively by this experiment. Several experiments were next made with the whole bile in which the colloid strength was increased by the addition of bland colloidal substances or diminished by diluting the bile with saline solution.

Experiment 9. 11. 13. 05. Two dogs of equal size. 12–1 P.M. Brown mongrel: 2 c.c. bile plus 2 c.c. 5 per cent. agar-agar. Mixture cooled to 40° C. Duct tied. No immediate effect on pancreas. White cur: 2 c.c. bile plus 2 c.c. salt solution. Duct tied. No immediate action on pancreas. 11. 14. 05. Both dogs still sick; the brown less prostrated than the white. 11. 15. 05. Both on feet; chloroformed, 11 A.M. Autopsies Brown (bile plus agar-agar). Peritoneum clear; no fat necrosis except spot or two just beside seat of operation. Corresponding to injected area of gland and extending slightly beyond, swelling and hæmorrhage of slight degree. No visible necrosis. The remainder of the organ appears normal. White (bile plus salt solution). Peritoneum normal except for fat necrosis in mesentery. Marked fat-necrosis about the pancreas. The mid-portion of the gland, except 2 cm. of splenic end, shows marked swelling, œdema, hæmorrhage, and fat necrosis.

The striking difference in the lesions caused, on the one hand, by bile of high colloidal quality, and, on the other, by the same bile in which the normal colloidal strength was reduced, is well brought out by this experiment. The protection afforded by colloidal substances may extend even further, so that the pancreas may entirely escape injury from a quantity of bile salts which, without such protection, is capable of causing severe lesions.

Experiment 10. 11. 17. 05. Two dogs of equal size. 11 – 2 A.M. Male: 2.5 c.c. bile + 2.5 c.c. 5 per cent. agar-agar. Duct not tied. Female: same mixture injected; duct tied. 11. 20. 05. Both dogs appear well. Female chloroformed. Peritoneum normal. No fat necrosis. Mid-portion of gland, receiving injection, moderately swollen and slightly œdematous, and on section slight hæmorrhage in tissue. No necrosis of parenchyma or fat. 11. 25. 05. Male chloroformed. The injection passed into the duodenal half and very little into the splenic parts of the gland. The peritoneum, excepting pancreas, normal. No fat necrosis anywhere. The injected end of the pancreas is white and glistening. the surface and section being granular. The granules include lobules and areas below lobules in size. The duct is patent. No evidence of hæmorrhage or necrosis.

It is clear from this experiment that by increasing sufficiently the colloidal strength of the mixture the bile can be made to act slightly upon, or be entirely prevented from causing acute lesions in, the pancreas. This experiment also suggests that, possibly, when the action is greatly diminished so that acute lesions fail to appear, the gland is yet sufficiently injured to respond with chronic productive lesions. Some years ago I showed[5] that in the dog chronic pancreatitis, especially a focalized kind about acute lesions, easily develops. But the present experiment does not prove that the bile modified and reduced by agar-agar sets up alone the chronic lesions, for should the agar-agar not be easily eliminated from the ducts it would act as a foreign obstructive body and in this way itself cause chronic inflammation. Indeed, I have found that if a three-per-cent. suspension of agar-agar is injected into the duct in sufficient quantity it will cause, at the end of a week, a well-marked sclerosis. On section of the gland in these cases remains of the agar-agar can be found in the tissues.

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[5] Flexner, *Johns Hopkins Hosp. Reports*, 1900, ix, 743; Flexner and Pearce, *Univ. Penna. Med. Bulletin*, 1901, xiv, 193.

That the biliary constituents, suspended in agar-agar, can pass out and come into relation with the gland tissue is shown to the naked eye by the loss of color which the agar suspension undergoes in the substance of the pancreas. This interchange of fluid between the gland and the agar suspension is a slow one relatively; and upon the rate of diffusion and the consequent degree of concentration of the biliary salts at any one time in contact with the parenchyma will depend, probably, the extent of the lesions.

Experiment 11. 11. 27. 05. Two dogs of equal size. 11–12 A.M. Brown: 2 c.c. bile plus 3 c.c. agar-agar. Injected duodenal half of gland. Black and white: 2 c.c. bile plus 6 c.c. agar-agar. Injected largest part of gland. 12. 4. 05. 11 A.M. Chloroformed. Brown dog: The injected portion of the gland is swollen and shows diffuse hyperæmia and punctiform hæmorrhages. The consistence is increased in this part, and the surface is granular. On section, the surface shows small mottled areas of hæmorrhage, fine yellowish, intra-acinar points of degeneration, and two larger softened areas of necrosis. The splenic half of the gland is kinked and at the angle an extraglandular cyst the size of a marble, containing clear fluid, has formed. The duct is patent until the bend is reached. It can be followed no farther. Beyond and about the cyst the gland is sclerosed. Black and white dog. Entire organ granular, sclerosed, and contracted. No acute lesions or fat necrosis. Main duct dilated slightly. In some parts the agar-agar has not been removed. The bile staining of the agar-agar has wholly disappeared.

This experiment calls for no discussion since it shows that with a sufficiency of colloid present the acutely injurious action of the bile can be prevented. It does not, however, settle the question of the relation of the chronic lesions to the diminished action of bile salts since the agar-agar itself remained, in the one case, to act as a foreign body. Hence in the next experiment colloids were chosen which were free of the objection that they were capable of congealing in the ducts.

Experiment 12. 12. 2. 05. Two dogs of equal size. 12 M. to 1 P.M. Buff mongrel: Bile 2 c.c. plus mixture of mucin and nucleoproteid[6] 4 c.c. Injection entered duodenal half of gland. Hairy mongrel: Bile 2 c.c. plus 20 per cent. gelatine 4 c.c. Injection entered splenic half of gland. Ducts not tied. The first dog quickly recovered, the second never really recovered, from the operation. Chloroformed, 12. 9. 05. Autopsy: Buff dog: The entire duodenal half of the gland and a small part of the middle portion are swollen, white in color, and firm in consistence. The remainder of the splenic portion of the pancreas is normal in consistence and appearance except for a vivid congestion. The line of demarcation between the swollen and normal gland is sharp. No evidence of acute lesion and no fat necrosis exist. Duct not dilated. Hairy dog: At seat of operation fat necrosis in the pancreas. The splenic portion of the gland except the tip is swollen moderately and firm. The serosa of the tip contains small hæmorrhages, and the injected portion, on section, is the seat of marked necroses in the parenchyma and interstitial tissue. This part and the duodenal end are both somewhat sclerosed.

From these experiments the conclusion can be drawn that the suspension of bile in a bland mixture of high colloidal strength protects the pancreas from the immediate and acutely injurious action of the biliary salts. But besides this conclusion another can be drawn, namely, that a relation exists between the nature of the suspending colloid and the reaction excited in the pancreas. If, for example, the colloid is readily attacked by the pancreatic juice and the density of the mixture reduced, then the injurious salts may still be brought into such mediate relation with the gland as to produce moderately severe acute lesions: this would appear to be the case with gelatine. But with agar-agar and the mucin-nucleoproteid mixture, which are acted upon little if at all by the pancreatic secretion, the protection is so effective as to eliminate wholly gross injury to the gland tissues.

That this explanation is the correct one is further proved by the next experiment, in which gum arabic was employed as the diluent.

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[6] A syrupy suspension of tendo-mucin and spleen nucleo-proteid was made in water by careful neutralization with $\frac{1}{10}$ N. sodium hydrate by Dr. Levene.

Experiment 13. 12. 14. '05. Two medium-sized dogs. 3.30 P.M. Hairy mongrel: Bile 2.5 c.c. plus gum-arabic suspension 2.5 c.c. Black terrier: Bile 2.5 c.c. plus saline 2.5 c.c. Ducts not tied. Both dogs up early next morning, but the first still very sick. About noon he refused to rise in his cage; dead, 4 P.M. Autopsy. The injection entered the duodenal half of the gland, which showed no marked inflammation, and no hæmorrhage or fat necrosis. There was slight swelling of the gland and a few necroses in the fat of the mesentery about the ducts. The cause of death of this dog is therefore not wholly clear The intestinal contents were blood-stained, and a few small broncho-pneumonic foci existed in the lungs. The terrier was chloroformed. Disseminated fat necrosis existed throughout the peritoneal cavity; it was very marked about the pancreas. The injection entered the mid-portion and splenic end of the gland. Fully two-thirds of the gland was the seat of marked acute inflammation associated with hæmorrhage. A blood clot of considerable size existed in the pancreatic and peripancreatic tissues about the middle of the gland.

This experiment is convincing in showing that by the addition of a bland colloid the action of bile on the pancreas can be greatly reduced or even entirely prevented; and it is also useful in showing that by reducing the colloid strength of normal bile by the addition of salt solution its injurious action can be considerably enhanced.

It would seem as if these conditions might be simulated in human pathology in instances in which the composition of the bile was modified by obstructive and inflammatory processes. The changes which imprisoned bile undergoes are well known. The loss of the diffusible salts and increase in muciginous constituents, which occur on obstruction of the ducts, and the accumulation of albuminous products, which takes place in inflammation, would provide exactly the theoretical conditions for the production of subacute or chronic lesions of the pancreas, rather than acute ones, provided this bile had been diverted into the pancreatic duct. If the conditions of these experimental lesions occur in human beings it seems not impossible that in this way may arise certain instances of partial or independent sclerosis of the pancreas which up to this time have received no adequate explanation.[7]

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[7] Perhaps it may be worth while to indicate that a mechanism exists in nature, comparable with, if not the equivalent of, the syringe, for driving a colloidal suspension of considerable thickness into the pancreas. This mechanism is the

That biliary salts should act as poisons for pancreatic tissue and blood-vessels is, in view of the established cytolytic property of the salts for various kinds of cells[8]—blood-corpuscles, amœbæ, infusoria, muscle, nerve, ciliated epithelium, liver cells,—not remarkable. Since this power is so great and can be exerted against the liver itself some restraint would seem to be required in nature. This restraint, apparently, can be supplied by the colloids in and about the cells. It is, however, probable that all parenchymatous cells are not equally susceptible to injury by the bile salts, and the liver cells are stated by Rywosch to be especially resistant.

When one reflects upon the mechanism of the restraining action of colloidal substances upon bile salts, one naturally turns to the consideration of physico-chemical notions for an explanation of the effects observed.[9] Bile salts exhibit a low surface tension and hence diffuse readily. They tend therefore to pass quickly into a surrounding fluid medium; and in the case of the pancreas soon find themselves in contact with tissue cells and blood-vessels. The latter immediately respond to their presence by hyperæmia and extravasation; and the injurious action is soon extended to the pancreatic cells. The colloids, on the other hand, are composed of large molecules of high surface tension. When the two sets of substances are mixed in solution, the molecules of the salts are spread out over those of the colloid, whereby mechanical conditions producing decrease in diffusion velocity of the salts are brought about.[10] In order that severe injury may be inflicted upon the pancreas, a certain concentration of the salts must exist in the fluids in immediate relation with the tissues; and as these fluids are in process of constant change, this concentration must be reached in a unit of time. Any

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gall-bladder and ducts, the muscular contraction of which might easily suffice for this purpose under conditions in which the biliary and pancreatic ducts had been, through pathological conditions, made continuous

[8] See Rywosch, *Inaugural Dissertation.* Dorpat, 1891.

[9] In view of the union of bile salts with proteids perhaps some account should be taken of this property.

[10] I am indebted to Professor Ostwald for this explanation. It was transmitted to me by the kindness of Dr. C. A. Herter.

marked reduction in diffusion velocity will, therefore, tend to reduce the action of the salts. Should the injury fall below a certain degree, and the action be prolonged, conditions favoring the production not of acute but of subacute or chronic lesions might be inaugurated. Moreover, the colloidal substances themselves while bland may not be without effect in causing new growth of connective tissue. For if the intrapancreatic pressure is too low to remove readily the colloid because of the higher friction which it exerts on the ducts, obstructive atrophy and sclerosis would result. But while this factor must be considered both in the experiments on dogs, and under similar hypothetical conditions in man, it can have no part in explaining the absence of the acute experimental lesions.

The rate of diffusion of crystalloids of low molecular weight into colloids (agar-agar) would appear, from Voightländer's[11] experiments, to be little lower than in water. Whether the same holds true for such large molecules as compose the bile salts I do not know; and I do not know, by direct experiment, whether a large molecular crystalloid, enclosed in a colloidal solution, would diffuse readily into a solution as complex as lymph. The experiments related in this paper would tend to speak against such ready diffusion. But it will be necessary to make the conditions of study simpler than those of these experiments before a final comparison of all the facts mentioned can be made.[12]

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[11] *Zeitsch. f. physik. Chemie*, 1889, iii, 316.

[12] Experiments relating to these questions are being pursued.

# ON THE CARBOHYDRATE GROUP IN THE NUCLEOPROTEID OF THE SPLEEN.

BY P. A. LEVENE AND J. A. MANDEL, NEW YORK.

(*From the Rockefeller Institute for Medical Research, New York.*)

By the term nucleoproteids is designated that class of substances which on cleavage yield, besides the usual components of the proteid molecule, phosphoric acid, purin bases, pyrimidin derivatives, and carbohydrates. While in recent years much progress has been made in our knowledge of the basic constituents of nucleoproteid, our knowledge of the carbohydrate moiety of the molecule has remained very imperfect. The present note deals with the results of our further investigation of the proteid derived from the spleen; but it is our intention to study, in a similar manner to that mentioned here, the proteids from other organs. We have succeeded in obtaining from the spleen a substance presenting the properties of glucothionic acid—that is, a nitrogenous body composed of sulphuric acid and carbohydrate.

Hitherto substances of this class had been obtained by Moerner and by Schmiedeberg from cartilage, by Krawkaw and Odi from amyloid, by one of us (Levene) and Gies and his co-workers from mucoids and mucin. Levene had also shown it to exist in the parenchymatous organs, a fact later extended by the present writers.

In the present study the nucleoproteid of the spleen was prepared in the usual manner by boiling the organs with water and acidulating the extract with acetic acid. The precipitate was washed by decantation and extracted with alcohol or with alcohol and ether. The air-dry product contained two per cent. of phosphorus. This proteid was subjected to the treatment already described by Levene[1] for obtaining glucothionic acid.

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[1] *Zeitsch. f. physiol. Chem.*, 1903, xxxvii, 400.

By this means a substance has been obtained which, while not absolutely free from nucleic acid, yet contains (one sample tested) 8.45 per cent. of nitrogen and 3.19 per cent. of sulphur. This body reduces Fehling's solution only after previous hydrolysis with mineral acids, and gives a pronounced reaction for pentose with orcin-hydrochloric acid. On cleavage of the proteid itself with dilute mineral acids a substance presenting the same properties was obtained. The proportion of sulphuric acid was, however, much lower.

Two interpretations of this finding occur to us. First, substances commonly designated nucleoproteid are composed of two distinct substances—mucoid and true nucleoproteid. Second, they contain in their molecule a glucothionic acid. We are at present engaged upon a further study of this problem.

# ON A CERTAIN CRYSTALLINE BODY OBTAINED ON THE PROLONGED DIGESTION OF GELATINE.

By P. A. LEVENE, New York.

(*From the Rockefeller Institute for Medical Research, New York.*)

In course of a study of the products of tryptic digestion of gelatine the writer demonstrated the formation of prolin.[1] While preparing a large quantity of prolin recently a crystalline substance was obtained which differed in its properties from the body sought. This substance is soluble in absolute alcohol and readily crystallizes from its solution in this solvent. It is precipitated by phosphotungstic acid, but is not precipitated by iodide bismuth-iodide potassium. Thus far an insoluble platinum salt has not been obtained; nor has a salt been formed with copper oxide. A picrate was prepared by means of an alcoholic solution of picric acid. The substance tastes strongly bitter and gives a pronounced test for pyrrol. The melting point of the pure substance is 182°–185° C., and of the picrate, 165°–167° C. The elementary composition is as follows: $C = 53.98\ \%$; $H = 6.67\ \%$; $N = 18.51\ \%$; $O = 20.84\ \%$. The calculated formula is $C_7\ H_{11}\ N_2\ O_2$. $C = 54.19$; $H = 7.09$; $N = 18.09$; $O = 20.63\ \%$. The formula calculated for $C_7\ H_{10}\ N_2\ O_2$ is $C = 54.55$; $H = 6.49$; $N = 18.18$; $O = 20.78\ \%$. A nitrogen estimation of the picrate gave 18.58 %. As calculated for $C_7\ H_{10}\ N_2\ O_2.\ C_6\ H_2\ (NO_2)\ OH$ the nitrogen is 18.27 % and as calculated for $C_7\ H_{10}\ N_2\ O_2.\ 2C_6\ H_2\ (NO_2)_3\ OH$ it is 18.30 %.

Hence it is probable that the composition of the substance is $C_7\ H_{10}\ N_2\ O_2$, and that it is in some way related to prolin. Further investigation into the nature of this body is in progress.

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[1] *Zeitsch. f. physiol. Chem.*, 1903, xli, 99.

# GASTRIC ULCERS IN RABBITS FOLLOWING RESECTION OF THE PNEUMOGASTRIC NERVES BELOW THE DIAPHRAGM.

BY W. OPHÜLS, M.D.

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PLATE XI.

The experiments reported in this paper were suggested by an article published by Van Yzeren,[1] one of Talma's assistants, in 1901. Van Yzeren cut both vagi in twenty rabbits below the diaphragm and observed the development in ten of chronic ulcers, usually single, in the pyloric region of the stomach. His results appear more remarkable still when we take into account that of the ten rabbits without ulcers, seven lived five days and less (five only one day), whereas the first ulcer was found in a rabbit that had lived seven days. Of the rabbits examined later, three only did not show ulcers (one examined on the ninth, one on the tenth, and one on the forty-seventh day). The last rabbit was examined 289 days after the operation and showed an ulcer still present.

Van Yzeren was preceded in this work by two Italian investigators: Lorenzi and Saitta. In 1893, Lorenzi[2] reported that after resection of the vagi either at the neck or below the diaphragm in rabbits, hemorrhages were frequently observed in the mucous membrane of the stomach and in some cases from these, when the animals survived longer than twenty-four hours, hemorrhagic erosions developed. In 1900, Saitta[3] performed bilateral vagotomy at the neck in sixteen rabbits, in which he treated as well as possible the resulting disturbances in respiration and heart action. Three of the rabbits died on the second day and showed normal stomachs, three died on the eighth to the tenth day and showed black ecchymotic spots in the mucosa, and the remaining four died on the fifteenth to the twentieth day, showing hemorrhages and erosions in the mucous membrane. In four rabbits that had been operated upon in a similar manner and had been given 3 per cent. hydrochloric acid solution by mouth, he found multiple ulcers.

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[1] Van Yzeren, "Die Pathogenesis des chronischen Magengeschwürs," *Zeitsch. f. klin. Med.*, 1901, xliii, 181.

[2] Lorenzi, "L'influenza del sistema nervoso nella patogenesi dell' ulcera dello stomaco," *Rassegna di Sc. med.*, 1893.

[3] Saitta, "Contributo alla patogenesi dell' ulcera gastrica," *Gaz. degli Ospedali Milano*, 1900, xxi, 599.

In 1901, dalla Vedova[4] studied the influence of disturbances in innervation on the gastric mucous membrane of dogs. His experiments were not confined to the vagus nerve, but included the ganglia cœliaca and the left splanchnicus magnus. The nerves were either resected, or alcohol, which, according to him, has the same effect, was injected into them. In six instances he resected the dorsal branch of the vagus below the diaphragm or injected alcohol into it without result. In twelve animals he succeeded in removing the left or both ganglia cœliaca. Positive results were observed in five of the latter. He removed the left splanchnicus magnus successfully in ten dogs, with nine positive results. These animals lived from one to sixty days. In all the positive cases but two he found hemorrhages and hemorrhagic erosions, and in the two remaining he found larger hemorrhagic, funnel-shaped ulcers. The last occurred after resection of the ganglion cœliacum, the animal living three days, and after injection of alcohol into the left splanchnicus magnus, the dog surviving the operation eighteen days.

The most recent paper on the subject, which is quite full, was published by Donati.[5] Donati resected both vagi below the diaphragm in nine dogs and eleven rabbits, extirpated the plexus cœliacus in eleven dogs and five rabbits, and removed both of these at the same time in one dog. He also resected both vagi at the neck in six dogs, but these animals lived only four to five days. He resected one vagus at the neck in three dogs, the animals being killed between the thirtieth and sixty-fifth day; and in another dog he resected one vagus at the neck and later the other one, the animal surviving eighty-one days. In all these experiments the results were entirely negative. Donati gives a very complete review of the literature, inclusive of the experiments made by physiologists in order to ascertain the function of the gastric nerves, and points out that as far as lesions in the mucous membrane of the stomach are concerned the results have been most contradictory. From his review of the literature and from the result of his own experiments he comes to the conclusion that very probably the hemorrhages, erosions, and even ulcers, described by other observers, were entirely accidental, and that disturbances in innervation have no effect whatever upon the mucous membrane of the stomach.

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[4] dalla Vedova, "Ricerche spirimentale sulla patogenesi dell' ulcera gastrica," *Arch. ed atti della soc. ital. di chir.*, 1901; *Archiv für Verdauungskrankh.*, 1902.

[5] *Archiv für klin. Chirurgie*, 1904, lxxiii, 908.

A short notice of the same work also appeared in the *Zentralblatt für Chirurgie*, 1904, xxxi, 346.

In spite of Donati's work it seems difficult to understand how the results of Van Yzeren could be due entirely to accident. Even granted that hemorrhages and erosions are comparatively frequently found accidentally in the gastric mucosa of animals, especially in rabbits, and my own experience leads me to be certain of this, still typical chronic ulcers have, at least as far as I am aware, never been described as occurring spontaneously in these animals. This point seems all the more important as rabbits are so very commonly used for experimental purposes and on that account carefully examined. In the opinion that chronic ulcers of the stomach do not occur spontaneously in rabbits I am confirmed by observations made on a considerable number of controls in the course of my own experiments on this subject.

In these experiments I have confined myself entirely to rabbits and have always resected both vagi below the diaphragm. The operation is easily performed. The animals are anæsthetized with ether, an incision is made along the free margin of the ribs on the left side, and the stomach is exposed and drawn downwards. After tearing through a thin peritoneal fold, which runs from the stomach to one of the lobes of the liver, the lower end of the œsophagus, which passes quite a distance through the diaphragm, is exposed; the nerves can then be easily found and a piece of about ½ cm. in length resected without producing any hemorrhage to speak of. If the anæsthetic is carefully given the animals recover very rapidly from the operation, and with fairly careful antiseptic precautions one should not lose any of them by accident. The animals operated on were examined at varying intervals. They do not as a rule show any bad symptoms as a result of the operation and remain in a fairly good state of nutrition. In a few instances only was a considerable degree of emaciation noticed, although not necessarily in those animals in which gastric ulcers developed. A careful examination of the site of operation was made in all cases in order to determine whether both vagi had been cut and, also, in order to exclude any complications resulting from a possible infection at the point of operation. In the first days following the operation one notices very little reaction. Later, a small amount of dense cicatricial tissue develops in which the cut ends of the vagi are embedded and which, on the other side, adheres to the cardiac end of the lesser curvature of the stomach. The amount of cicatricial tissue found was no greater in the animals developing ulcers than in the ones which gave negative results. I have never been able to find any macroscopic evidence of regeneration of the nerves.

Thirty rabbits were operated upon in this way and the results are shown in Table I, page 185. The transverse fold mentioned is one which is plainly seen when one examines *in situ* the interior of the stomach of a rabbit through an incision along the greater curvature. Several smaller and one large transverse fold are then noticed at the lesser curvature between cardia and pylorus and a little nearer to the pylorus. This fold becomes narrower and lower on both sides and disappears before it has reached the greater curvature.

There were then six positive results in thirty experiments, or, if we exclude those animals examined before the twenty-fourth day, at which day the first ulcer was found, six positive results in eighteen experiments were obtained. It is at once apparent that in my series the ulcers developed much less regularly than in Van Yzeren's series. Whenever they were found, however, they corresponded very closely to Van Yzeren's description. They were undoubtedly chronic ulcers, as was shown by the marked induration at the base; they appeared of round or oval form, with sharp edges and smooth bottom. All the ulcers which I obtained were single, while Van Yzeren observed several instances in which they were multiple. Since Van Yzeren neglected to give any pictures of the ulcers I have photographed one of the larger ones, which is shown in natural size in Fig. 1. This photograph also shows well the relation of the ulcer to the transverse fold. Sections were made of two of the larger ulcers. The ulceration extended, in one case, into the muscle, and in the

TABLE I.

| No. of Rabbits Examined. | Day after Operation. | Result. |
|---|---|---|
| 1 | 3d | Negative. |
| 2 | 5th | " |
| 2 | 7th | " |
| 1 | 8th | " |
| 1 | 10th | " |
| 1 | 11th | " |
| 2 | 15th | " |
| 1 | 17th | " |
| 2 | 18th | " |
| 1 | 24th | Transverse oval chronic ulcer about 8 x 10 mm. on summit of fold. Sharp edges, smooth bottom. |
| 1 | 26th | Negative. |
| 1 | 34th | Small chronic ulcer on fold. |
| 1 | 39th | Negative. |
| 1 | 42d | " |
| 1 | 45th | " |
| 3 | 50th | In one, small chronic ulcer 2 mm. from pylorus. |
| 1 | 60th | Chronic oval ulcer 7 x 9 mm. on summit of fold; sharp edges. |
| 2 | 64th | In one, nearly healed ulcer with much induration on summit of fold. |
| 4 | 71st | Negative. |
| 1 | 88th | Small chronic ulcer on anterior wall in pyloric region, but near fundus. |

NOTE.—Of thirty rabbits operated upon and examined six showed ulcers of the stomach. No ulcer found earlier than 24th day. Eighteen rabbits examined after 24th day; hence ulcers developed in 33 per cent. of these rabbits.

other, of which a microphotograph is given (Fig. 2), through the muscle into the subperitoneal tissue. In both ulcers there was a considerable development of cicatricial tissue at the base, which was covered with a thin layer of necrotic material including fibrin, blood, remnants of food, etc. The mucous membrane at the edge, as may be seen in the microphotograph, may show a considerable hypertrophy and project over the ulcer. The larger arteries and veins near the ulcer were carefully examined in numerous sections; they were found to be entirely normal.

During the course of the experiments I have examined the stomachs of a large number of normal rabbits. I have several times found small hemorrhages, and in one case I found a hemorrhagic erosion with complete destruction of the mucous membrane over an area of about 2 mm. in the center, on the summit of the above described fold, but the process was evidently quite recent. At the bottom of the defect there was a narrow zone of necrosis and a few leucocytes could be seen in the tissues beneath, but no other reaction was visible. I have examined certainly as many normal rabbits as operated ones. All our rabbits are kept together in a large rabbit pen, to which the animals operated on were returned after they had sufficiently recovered from the operation. Hence all the rabbits eat the same food and are otherwise kept under identical conditions. The rabbits used in the experiments were taken from the same lots as were those which were examined without operation.

It could therefore hardly be a matter of chance that the chronic ulcers were found only in the animals operated upon which survived the operation twenty-four days or longer. I have already mentioned that if chronic ulcers in rabbits occur at all spontaneously, they must be very rare indeed. I believe, therefore, that in spite of Donati's eleven negative experiments we are safe in concluding that resections of both vagi below the diaphragm in rabbits may be followed by the formation of peculiar chronic ulcers beginning in the mucous membrane of the stomach, but that this result is not either constant or remarkably frequent.

The question arises as to what relation exists between the section of the nerves and the production of the ulcers. We should enquire, naturally, first of all, of the function which the vagus nerve exerts upon the stomach and what the disturbances of this viscus are after the nerve is cut.

It can hardly be doubted that the motions of the stomach are to a certain extent under the influence of the vagus nerve. That this is true can be plainly seen upon stimulating electrically or otherwise the two vagi below the diaphragm. The result is a strong contraction of the muscle of the stomach, which seems to be similar to the normal contractions, but more vigorous; and, if the stimulation is not so strong as to produce a tonic contraction, the contractions are repeated more frequently. It is also claimed by some that the vagus nerve contains inhibitory fibres. If, on the other hand, the nerve is cut, it has been found that the movements of the stomach are retarded, but that otherwise they continue unchanged. In my experiments I noticed in the first weeks a marked dilatation of the stomach. The normal capacity of an ordinary rabbit's stomach is about 100 to 120 c.c. The capacity was increased often to 180 c.c. or more. At the same time in these instances it was noticeable that the elastic properties of the gastric wall were unaltered. With the increase in contents there was a marked rise in pressure within. The dilated stomachs therefore felt very hard to the touch, and on that account could be easily palpated through the abdominal wall in the living animal. The operated animals seem somehow to overload their stomachs. I believe that it is this condition that misled Van Yzeren and led him to assume that tonic cramps of the muscle occurred after the resection; at least I have never seen any evidence of the occurrence of such cramps. In all my instances the hardness of the organ was plainly accounted for by overfilling. This disturbance of motion seems in course of time to be compensated, for in all the animals examined late the stomach had returned to its normal size and the tension of the wall had become normal.

On the whole, the vagus seems to have very little influence upon the secretion of gastriç juice. Pawlow, it is true, has demonstrated that the central reflex for the secretion of the gastric juice passes through the vagus, because in his dogs the secretion of gastric juice ordinarily taking place when they see tempting food is missing when the vagus is cut. But our rabbits seem to have done well in the absence of this central reflex. Direct stimulation of the nerves below the diaphragm is followed by the appearance of considerable gastric juice upon the mucous membrane, which phenomenon can be repeated several times after the secretion has been wiped away. Still, it is of course impossible to decide whether an actual secretion takes place as a result of the stimulation or whether preformed gastric juice is simply squeezed out of the mucous membrane by the contraction of the muscular elements in it. In any case the proper secretion of gastric juice does not seem to have been disturbed materially in our rabbits by the section of the vagi. The contents of the stomach of the operated animals had absolutely normal appearances and the chemical examination did not reveal any constant changes. The result of the chemical examinations made by Töpfer's method in several rabbits is given in Table II. The figures represent the number of cubic centimeters of decinormal solution of sodium hydroxide necessary to neutralize 100 c.c. of gastric juice. The latter was obtained by adding an equal volume of distilled water to the gastric contents and filtering. The figures obtained with the filtrate were doubled. The method may not be entirely accurate, but it gives comparable values. It was of course impossible to obtain the gastric juice always at the same stage of digestion, which probably accounts for the rather wide discrepancies in the figures.

Another possibility that suggests itself as an explanation of the formation of the ulcers after resection of the vagi is the disturbance of those protective mechanisms which seem to exist in the gastric and intestinal mucous membranes in order to prevent undue injury to them from their contents. These mechanisms have been described by Exner[6] and by Müller.[7] These authors found that mechanical irritation of the mucous membrane of the intestines and of the stomach in dogs and cats produces, first, an anæmia at the point of irritation, which is followed by a small dimple resulting from the contraction of the muscularis mucosa at the spot. Exner had already noticed that the same phenomenon occurs in the intestines of animals shortly after death, or even when pieces of intestine have been cut out. Müller sometimes observed a disappearance of the phenomenon in the stomach of cats after resection of the vagi below the diaphragm, but at other times there was no change.[8]

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[6] Exner, "Wie schützt sich der Verdauungstract vor Verletzungen," *Arch. f. Phys.*, 1902, lxxxix, 253.

[7] Müller, "Beitrag zur Kenntnis von den Schutzeinrichtungen des Darmtractes," etc., *Arch. f. Phys.*, 1904, cii, 206.

TABLE II.

| | Total Acidity. | Free HCl. | Loosely Comb. HCl. | Organic Acids and Acid Salts. |
|---|---|---|---|---|
| Normal rabbit | 124 | 96 | 4 | 24 |
| " " | 88 | 76 | 8 | 42 |
| " " | 148 | 128 | 16 | 4 |
| " " | 132 | 132 | | |
| Rabbit 5 days after operation | 92 | 72 | 4 | 16 |
| " 11 " " " | 96 | 48 | 16 | 32 |
| " 42 " " " | 100 | 80 | | 20 |
| " 50 " " " | 112 | 72 | 8 | 32 |
| " 50 " " " | 132 | 104 | 16 | 12 |
| " 50 " " " with small ulcer in pyloric region | 88 | 48 | 8 | 32 |

I was led to make similar experiments upon the mucous membrane of the stomach of rabbits by the fact that the disturbances arising from section of the vagus at the neck are commonly ascribed partly to interference with normal reflexes, such as, for instance, the laryngeal reflex. The gastric mucous membrane of rabbits also responds to mechanical irritation in a definite and similar manner to that described for cats and dogs by

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[8] Both Exner and Müller describe these phenomena as due to reflex action. I have purposely avoided this term in the above description, because in spite of their arguments to the contrary it does not seem definitely settled whether they could not be the result of direct stimulation of the muscular elements in the mucous membrane. It is probably the chief if not the only function of the muscularis mucosæ to draw the mucous membrane away from points of mechanical irritation.

Exner and Müller. It is interesting to note that the response of the mucous membrane is much more prompt and marked in the region of the lesser curvature, which is the usual site of the ulcers. This difference is so distinct, that while in the fundus one notices hardly any effect on pricking or touching the mucous membrane with a blunt instrument, the anæmia and the motion in the mucous membrane are quite evident under similar conditions at the lesser curvature. As a result of the first tests in which I irritated the mucous membrane chemically, a form of irritation to which it reacts much less promptly, I had the impression that cutting of the vagus interfered with or completely abolished the reaction; but this opinion has not been borne out by my later experiments with mechanical irritation. Indeed it would seem as if the reaction was sometimes even more marked after the vagi were cut. The reaction also persists for a time after section of the vagi. Animals were examined at varying intervals after operation and no change in the reaction was noticed. In one case I had the opportunity of testing for the reaction in a stomach that was the seat of a chronic ulcer. No reaction could be elicited in the immediate neighborhood of the ulcer, but this was probably due to the immobilization of the mucous membrane by the chronic inflammatory process. A little farther away the reaction was weak, but undoubtedly present. It cannot therefore very well be any interference with this protective reaction that causes the ulcerations.

I believe that originally the ulcers are traumatic in origin, due to the presence of hard, more or less pointed, substances in the food. This explanation would account for their occasional or more frequent presence, depending on accidental circumstances, and serve to harmonize my results with those of Van Yzeren. Their position on the summit of a projecting fold is suggestive also of this mode of origin. The hemorrhages and erosions observed in the stomach of normal rabbits are also probably due to the same cause. It seems, however, very difficult to explain why the ordinary defects produced in this way heal rapidly, while after section of the vagi a tendency to development of chronic ulcers exists.

Van Yzeren bases his explanation of the origin of the ulcers on the occurrence of cramps in the muscle, which he claims to have observed. He believes that the cramps interfere with the blood supply of the mucous membrane and produce an anæmic necrosis at the point where the ulcer is to develop. He found confirmation of this theory in certain experiments in which he not only cut the vagi, but also made deep incisions into the muscle from the outside, or performed gastroenterostomy. If the latter operations, designed to relieve the cramps, were added, no ulcers developed. Quite apart from the fact that it seems more than doubtful whether such cramps actually occur, it is not easy to understand how they could produce a localized anæmic necrosis; and, even assuming that they do so, it still remains to be explained how such an obstacle to the usual rapid healing of defects could operate. Since the ulcers do not occur as regularly as Van Yzeren believed, his comparatively few experiments designed to prevent the occurrence of them by the above described means are, to my mind, hardly conclusive.

One could imagine that traumatism is more apt to occur when the stomach is overfilled and very tense, as is the case in the first week after vagotomy; and that the increased pressure of the contents might also stand in the way of a proper healing of any defect produced in this fashion. Unfortunately, for this supposition, the motor conditions seem to return to the normal after a little while. Still, it is a well-known fact that after chronic ulcerative processes have once been started, healing may be retarded though the pathological conditions that caused the ulceration be removed.

It seems necessary to discuss one other possibility, namely, that the ulcers belong to the so-called tropho-neurotic lesions; that is, that through the resection of the nerves trophic influences are removed which are necessary to preserve the normal resistance of the mucous membrane. I believe this explanation should be accepted only after all other possible explanations have been exhausted, and yet from the data on hand, it has certainly to be considered. There is something mysterious about the assumption of such tropho-neurotic influences that is somewhat contrary to our ordinary modes of reasoning; and still it cannot be denied that certain well-known facts could hardly be explained otherwise than on the basis of some such hypothesis.

I believe then that my experiments have shown that in rabbits there is some relation between the resection of the vagi below the diaphragm and the occurrence of chronic ulcers of the stomach. The experiments, I am sorry to say, have not shed much further light on the question of what this relation is. I also believe that it would be entirely premature to make use of these experiments in the explanation of peptic ulcer in man, although I acknowledge that the resemblance between the two processes is striking.

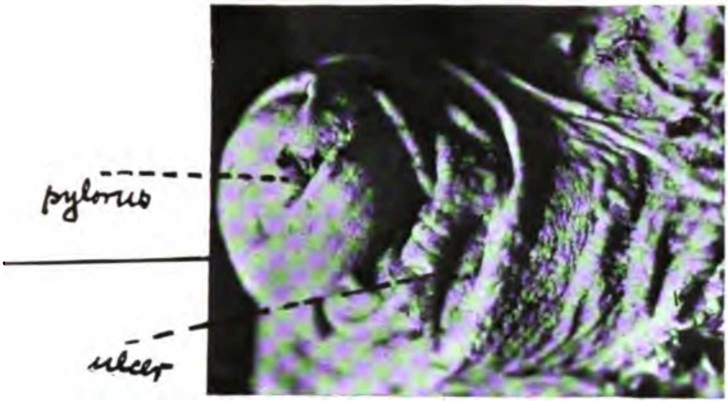

FIG. 1

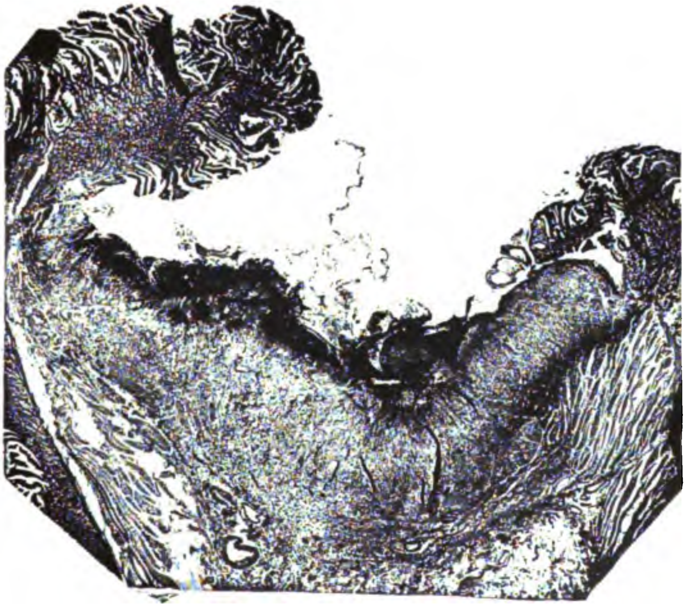

FIG. 2

# MORPHOLOGY AND PHYSIOLOGY OF AREAS OF LANGERHANS IN SOME VERTEBRATES.

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PLATES XII, XIII, XIV, AND XV.

Probably no organ or tissue of the body has been the subject of more thought or investigation than have the areas of Langerhans, especially during the last few years, and yet there are many questions that still remain unanswered. Since a complete review of the extensive literature on this subject would extend this paper beyond the limits that seem desirable, and since relatively exhaustive reviews of the literature have recently been given by Schulze, Ssobolew, Sauerbeck, and many others, it seems unnecessary to do more than call attention briefly to articles which deal with those phases of the subject with which my own work has been especially concerned.

As is well known, the islands were first described in 1869 by Langerhans and later by Laguesse, Opie, Diamare, v. Ebner, Gutmann, and many others. As to their development, Hansemann believes that they are mesenchymal in origin, having no connection at any time with the pancreatic tubules, but arising from the interstitial tissue. Küster and Pearce, investigating human embryos, and Laguesse from the study of sheep embryos arrived at the conclusion that the islands develop from the same anlage as the pancreatic glandular structures. These authors differ, however, somewhat in the details of their conclusions, since Küster states that the islands arise from the ducts, the anlagen, when first differentiated, showing central nuclei, homogeneous protoplasm with a tendency to a band-like arrangement of the cells and having capillaries between the bands of cells.

Pearce, on the other hand, believes that they develop from the pancreatic tubules and are at first solid and later vascularized, a reticulum developing still later. Laguesse concludes that they have originally the same entodermal origin as the glandular acini, but later they are transitory structures, developing from the tubules and changing back into the tubules.

On the general structure of these areas, investigators are practically agreed and the description given by v. Ebner expresses briefly the generally accepted ideas concerning their structure. He states that as a rule, but especially in man, the Langerhans islands are surrounded by a connective tissue sheath, which may be interrupted in places so that island cells and acinal cells are in immediate contact. The arrangement of the cells is peculiar because of their close relation to the large blood vessels, which he regards as venous. These are surrounded on all sides by the cells, like the blood capillaries of the liver lobule, the cells resting directly on the walls of the large blood capillaries without any membrana propria or adventitia capillaris between them. In man, occasionally, the area is divided into smaller compartments by strands of connective tissue and within these compartments again the capillary walls rest directly on the epithelial cells. As a rule, several rows of cells are found between two neighboring capillaries. To some extent in mammalia, but especially in amphibia, single rows of cells are seen between the capillaries, so that each cell is in contact with blood capillaries, on two sides. No excretory duct can be found between the cells. The cells are polygonal in shape, 9–12 μ in diameter (smaller than the gland cells), with very finely granular protoplasm which does not stain well with eosin. The nuclei are ellipsoidal, show a fine chromatin network, and nucleoli which are never so large as those of the glandular cells.

Harris and Gow distinguish three main types of islands in the different species of animals examined : (1) Those having no distinct cells but many small deeply stained nuclei, so that the areas appear not unlike lymphoid tissue. The most characteristic islands of this type are those of the guinea-pig. (2) Those in which the cell outlines are distinct, the cells non-granular and joined irregularly or forming a network. The areas of the armadillo, potto, and glutton are of this type. (3) Compound cell groups, in which each area is divided into smaller masses by strands of connective tissue. This type is well seen in the human pancreas and also in that of the eagle-owl. V. Ebner, however, states that he finds all these types in the same animal and thinks that the differences depend upon whether the capillaries are collapsed or distended with blood. Diamare (1899) has investigated the areas of Langerhans in teleosts, reptilia, mammalia, aves, and amphibia, finding that in all cases they consist of cords of epithelial cells separated by blood-vessels, but differing in the size and arrangement of the cell cords and in the development of the vascular rete. Pugnat, studying the pancreas of birds of many different species, finds large irregular areas consisting of small elongated cells with indistinct protoplasm and clear nucleus and regards them as lymphoid in nature. In 1904 Rennie published a most important communication concerning the areas of Langerhans in teleostei; in it he reports the results of his study of the areas in twenty-five species of osseous fishes, in all of which very large areas were found in the small islets of pancreatic tissue scattered along the abdominal vessels and among these he finds nearly constantly present a large, so-called "principal islet" a short distance in front of the spleen in the mesenteric fold between the portal vein and the mesenteric artery. Others were found almost constantly in many species. In *Lophius piscatorius*, this principal islet is very large and constant in its position and is independent of pancreatic tissue, being surrounded by a capsule of areolar tissue.

Regarding the connective tissue of the insulæ, Flint says, as a result of his investigations of the human pancreas by the Spalteholz method, that the connective tissue of the islands is definitely and characteristically arranged and sharply contrasted with that of the rest of the lobule. The islands are surrounded by a distinct fibrous capsule in relation with the alveolar framework. Septa or trabeculæ divide the area into smaller compartments containing the cells. His figure shows a septum passing through the center of the area and branches passing off from it to either side dividing each half into compartments of fairly uniform size. Laguesse, on the other hand, states that there is no connective tissue, but a thin, homogeneous layer, which may be thickened in places ("pseudo capsule"), and that a thin, amorphous sheath accompanies the principal vessel and may spread out over the capillaries. V. Ebner says that as a rule there is no connective tissue or membrana propria between the island cells and the capillaries.

As to the blood supply of the islands, while all agree that they are richly vascularized, there is some disagreement regarding the character of the vessels. V. Ebner regarded them as venous and was supported by Diamare. Kühne and Lea state that the vessels arise partly from capillaries and partly from arterioles. Hansemann finds only capillary connections, and Opie states that " the glomerularnet work is in very free communication with the smallest arteries and that apparently the blood supply is richer than that of other parts of the lobule." Laguesse states that generally a principal vessel enters the island (rarely an arteriole, generally a large branch continuous with an arteriole). In a very recent article, which is profusely illustrated, Pensa has reported the results of vascular injections of the areas of Langerhans in a large number of species of vertebrates. He finds the areas richly vascularized, mostly by a capillary network which is a continuation of the intertubular capillary plexus of the pancreas. In some animals, as birds, guinea-pigs, and dogs, the larger areas may show, in addition to the capillary vessel, a small afferent artery, breaking up into a capillary plexus within the island, the blood being then collected into a single efferent vein. He does not state, however, how he distinguishes the arteries from the veins, and while he states that the connection is usually purely capillary, his Fig. 2 Plate IV, seems to indicate that the connection with larger vessels is very common.

By the Golgi method, Pensa was able also to demonstrate a very rich network of nerve fibers in the areas, the nerves passing along the blood-vessels and also between the cells, and being distinctly different both in number and arrangement from the nerves of the acini.

Dogiel by the Golgi method, and Kühne and Lea and also v. Ebner by injecting the ducts, showed that the areas of Langerhans are not connected by permeable ducts to the excretory ducts of the pancreas, although Lewaschew believed that some of the injection mass penetrated within the island. His results are believed to be due to an extravasation of the injection mass.

Laguesse divides the areas of Langerhans of man with respect to their size into very small, less than 100 μ. in diameter; small, 100μ to 150μ; medium, 150μ to 200μ; large, over 200μ in diameter; and giant forms (very rare), over 400μ in diameter.

After examining ten human subjects, Opie determined that there was in the head of the pancreas an average of 0.366 areas to the square millimeter, 0.36 in the body, and 0.68 in the tail, those in the tail being therefore four times as numerous in the cubic millimeter as in other parts of the pancreas. Sauerbeck in twelve human subjects finds an average of one per square millimeter, while Laguesse in six subjects finds an average of a little less than one per square millimeter and regards anything less than 0.5 per square millimeter as a diminution. He states that the one-hundredth part of the pancreas is area of Langerhans.

As to the distribution of these areas, Opie states that they are four times as numerous in the tail as in any other part of the pancreas and are situated in the center of the lobule, at least in the cat, in which he finds one area in each lobule in the splenic portion of the gland. In man, he says the number is more variable.

My own investigations on the morphology and histology of the areas of Langerhans have been made (1) by reconstruction by the Born wax-plate method of areas of Langerhans from man, cat, rabbit, and rat, and also of injected preparations from cat; (2) by careful study of serial sections of injected and uninjected preparations of pancreas of man, cat, rabbit, rat, guinea-pig, frog, and bird. The tissues were fixed as soon as possible after the death of the animal, in most cases in saturated aqueous solution of bichloride of mercury, and imbedded in paraffin, and serial sections averaging 5μ in thickness were cut. These were stained in hæmatoxylin and eosin, Mallory's connective tissue stain, Weigert's elastic stain, and in some few cases in Heidenhain's iron-lac hæmatoxylin and in saffranin and *Licht-grün*. Some of the most satisfactory and instructive preparations were those in which there was some congestion so that there was a physiologic vascular injection. These were often more satisfactory for study of the structure than those injected with a gelatin mass, in which the cells of the areas were often more or less changed. For the injected preparations, the simple Berlin blue and carmine gelatin masses were used and also a double injection, the veins being filled with the Berlin blue or carmine gelatin, while the arteries were filled with a granular mass (cinnabar or ultra marine blue.) In this procedure it was difficult to prevent a rupture of the smaller vessels and an extravasation of the granular mass, but some areas of the pancreas often showed a good double injection. In the estimation of the size of the areas, sections near the center of the areas were measured for the first two dimensions, while the third was obtained by counting the number of sections of the series through which the given area extended and multiplying by 5*u*, the thickness of the sections. It is easy in nearly all forms to distinguish the central from the peripheral sections, not only by their size but also by the fact that in the center of each area the smallest cords of cells and the largest blood-vessels are found. The volume, as given, is never exact, since it is obtained by simply multiplying the three dimensions, together. However, it answers the purposes of comparision and any more exact method would be exceedingly difficult because of the irregular shapes of the areas.

In the *guinea-pig*, I found an average of 1.07 areas per square millimeter or 1.14 areas per cubic millimeter. As the average volume of these areas was .0091 cu. mm., about one hundredth of the pancreas consists of island tissue. The areas vary greatly in size and shape as may be seen from a comparison of the measurements of the following ten areas·

| | | |
|---|---|---|
| 1. | 0.40 x 0.32 x 0.285 mm.................. | 0.036 c. mm. |
| 2. | 0.13 [illegible] 0.30 x 0.120 mm.................. | 0.0047 c. mm. |
| 3. | 0.29 x 0.33 x 0.115 mm.................. | 0.01 c. mm. |
| 4. | 0.14 x 0.45 x 0.185 mm.................. | 0.012 c. mm. |
| 5. | 0.15 x 0,32 x 0-175 mm.................. | 0,0098 c. mm. |

| | | |
|---|---|---|
| 6. | 0.13 x 0.18 x 0.11 mm | 0.0026 c. mm. |
| 7. | 0.10 x 0.13 x 0.125 mm | 0.0016 c. mm. |
| 8. | 0.12 x 0.17 x 0.18 mm | 0.0037 c. mm. |
| 9. | 0.15 x 0.2 x 0.25 mm | 0.0065 c. mm. |
| 10. | 0.12 x 0.27 x 0.28 mm | 0.0098 c. mm. |

They are usually oval or elongated in shape with quite regular contour, rarely lobulated or even with marked projections. Near the periphery of the pancreas, the areas are relatively small and spherical or oval, while near the central portion of the gland in the neighborhood of the larger ducts, especially at about the junction of the splenic and middle thirds, the areas are large and prominent. In this region, especially large islands are occasionally found, analogous to the giant areas described by Laguesse in the human pancreas. These often seem to have no connection with the pancreatic acini, being surrounded by the fibrous connective tissue and adipose tissue around the duct. While they vary in shape, many of them are much enlongated, being several times as long as they are wide, some having been found measuring 0.9 to 1.0 millimeter in actual length and 115 to 120 μ in width. In this region also masses of lymphoid tissue are occasionally seen, easily differentiated from the islands by the different character of the cells and by the arrangement of the cells and blood-vessels. The number, position, and relations of these central areas are represented in Fig. 1, a low power drawing of one of the main ducts of the pancreas of a guinea-pig, with the surrounding connective tissue and blood-vessels. In this small mass of tissue, measuring 3.2 mm. in length, five relatively large areas are seen, and three others were just beyond the field of vision. A small amount of pancreatic tissue is seen in the section, but it has no close relation to the areas of Langerhans, which are distinctly encapsulated. Occasionally at the periphery of the pancreas in the adipose tissue of the surrounding mesentery, I have seen areas which appeared to be free. Usually by tracing such areas through the series of sections in which they occur, some few pancreatic tubules are seen in loose or close relation with them.

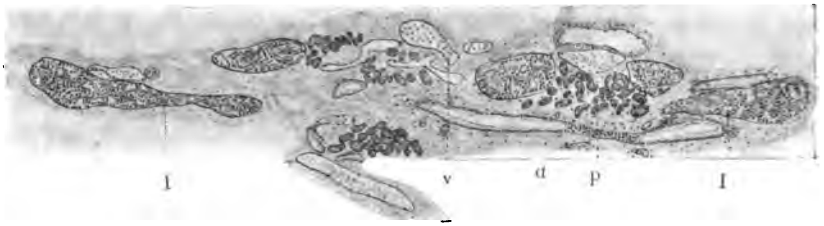


Fig. 1.—Large duct from pancreas of guinea-pig, showing surrounding connective tissue and blood-vessels with a small amount of glandular tissue and five relatively large areas of Langerhans. I, areas of Langerhans; p, pancreatic acini; v, blood-vessels; d, duct. × 25.

The discovery of such relatively isolated islands suggested to me the possibility that isolated areas might be found in the mesentery of some guinea-pigs, as in the teleostei, as shown by Rennie. Since the observation was made, however, only a small number of guinea-pigs have been at my disposal, and in these I have been unable to find isolated areas at any considerable distance from the pancreas, although, as in the teleostei, strands of island-containing pancreatic tissue accompany the vessels for some distance. With the exception of the relatively isolated areas above described, the insulæ of the guinea-pig's pancreas are in close relation to the pancreatic acini, being separated from them only by the membrana propria, which is often difficult to demonstrate, and sometimes for a distance by a capillary.

The cells are usually described as of two kinds, but to my mind, this classification scarcely covers all the cells found in the islands. (1) The great majority of the cells are, as has often been stated, polyhedral in shape, have homogeneous or nearly homogeneous protoplasm staining faintly with eosin, and have spherical or oval nuclei which are about the size of the nuclei of the acinal cells and show chromatin granules and one or two nucleoli. Many of the cells show distinct cell outlines. (2) There are a few cells with homogeneous protoplasm and very large faintly staining nuclei. (3) Small cells, with small, deeply staining nuclei occur. (4) Large cells with eosinophile granules in the protoplasm and with large, faintly staining nuclei are found. (5) Eosinophile cells with small, excentric, deeply staining nuclei are usually not very numerous and may be found either near the periphery of the island or in the central portion adjoining a blood-vessel. In well-fixed and well-stained sections, I have never found the syncytial appearance described by Hansemann or the masses of nuclei without cell differentiation described by Harris and Gow.

In every respect the structure of the central insulæ surrounding the duct is the same as that of the intralobular areas. The insular cells are arranged in irregular, sinuous bands, sometimes, especially in the central part, consisting of a single row of columnar cells with deeply staining and often quite large nuclei, sometimes of a double row of such cells, while in places, especially at the periphery of the area, the cells are grouped into small masses

The bands of cells follow the arrangement of the blood-vessels, but I failed to find any arrangement of blood-vessels or cords of cells which I could regard as characteristic for the species. In some sections, a larger vessel may be seen entering at one end of the area and passing through the center of the island, dividing it into two more or less equal portions; smaller branches passing off from the sides of this main vessel subdivide each half into larger or smaller compartments, giving a figure not unlike the figure and description given by Flint of the arrangement of the connective tissue in the human areas. Very little or no connective tissue, however, accompanies the vessels. In other sections, the vessel enters at one side soon dividing into numerous larger branches, which pass obliquely through the area in every direction, giving off many secondary branches which frequently anastomose by means of true capillaries.

The capsule, if such it may be called, is very delicate, and the walls of the insular capillaries seem to rest directly on the epithelial cords. When some shrinkage has taken place, however, the capillary occupies the center of a space which separates it from the epithelial cords, and in preparations well stained with Mallory's connective-tissue stain, delicate sheaths of connective tissue are seen to surround the blood-vessels; delicate lines of blue outline the cells, while a very thin capsule of connective tissue separates the area from the pancreatic acini. The amount of connective tissue in the areas is so much less than

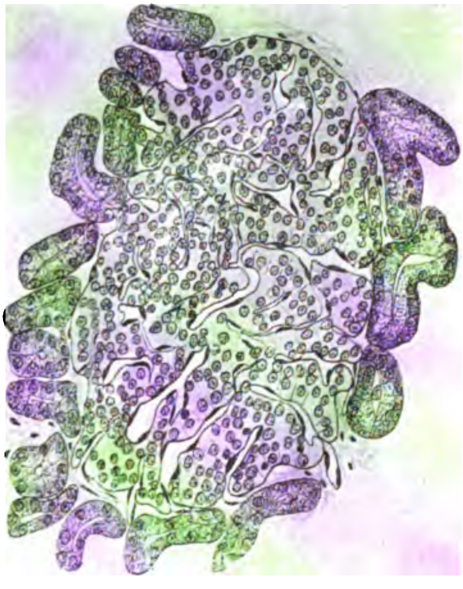


Fig. 2.—Area of Langerhans from pancreas of guinea-pig. Section through center of area. ×20.

that of the surrounding acini, and the arrangement is so different that the areas are sharply differentiated from the acini by the arrangement of the connective tissue alone. Fig. 2 shows a section through the center of a rather typical intralobular area of Langerhans of the guinea-pig. It is surrounded by pancreatic tubules except at one end, where the area reaches the periphery of the lobule and is limited by the interlobular connective tissue. The large columnar cells, single or double rows of which form the central cords of the area, and the more crowded cell masses at the periphery are well seen. These peripheral masses resemble somewhat the description given by Harris and Gow of the areas of Langerhans of the guinea-pig,—masses of nuclei, not differentiated into cells, and somewhat resembling lymphoid tissue—but the area as a whole is distinctly different.

In the *rat* also, the areas near the center of the pancreas are larger and more numerous than those near the periphery, but I have never, in the material at hand, been able to find the large isolated or nearly isolated central areas so characteristic for the guinea-pig. The measurements given below indicate to some extent the variations in size and shape of the areas of Langerhans in the rat.

| | | |
|---|---|---|
| 1. | 0.53 x 0.27 x 0.26 mm. | 0.037 c. mm. |
| 2. | 0.50 x 0.43 x 0.32 mm | 0.0688 c. mm. |
| 3. | 0.14 x 0.38 x 0.22 mm | 0.018 c. mm. |
| 4. | 0.22 x 0.23 x 0.40 mm | 0.02 c. mm. |
| 5. | 0.26 x 0.62 x 0.475 mm | 0.076 c. mm. |
| 6. | 0.16 x 0.25 x 0.30 mm | 0.012 c. mm. |
| 7. | 0.30 x 0.60 x 0.51 mm | 0.092 c. mm |
| 8. | 0.14 x 0.18 x 0.29 mm. | 0.007 c. mm. |
| 9. | 0.17 x 0.51 x 0.465 mm | 0 04 c. mm. |
| 10. | 0.15 x 0.18 x 0.21 mm | 0.0376 c. mm. |

The average size of these ten areas is .0376 c. mm. and, as the average number is 0.7 per mm. in the material at hand, about $\frac{3}{100}$ of the pancreas consists of insular tissue. It is somewhat difficult to be sure of counting areas correctly in the rat, since most of the areas are irregular and lobulated so that often areas may appear as two quite widely separate areas in one section, while in another they unite into one relatively large island. This can easily be seen from Plate XII, Fig. 1, which reproduces a model of a typical area of Langerhans from the rat's pancreas. This island of Langerhans is not nearly so large nor so lobulated as islands often appear, in tracing them through the series of sections in which they occur. It consists of two distinct lobules of insular tissue, well separated at the periphery, but joined at the center into a solid mass. The sponge-like appearance of these areas is well seen in the figure, since, as stated before, the relatively loose, band-like arrangement of the cells at the center is concealed to some extent by the more solid masses of small cells at the periphery, through which only the small capillaries and the few afferent and efferent vessels pass. In the rat, however, the cells at the periphery are to some extent arranged in irregular cylinders resembling tubules in contour, but having no lumen and having a very free communication with other cylinders. The cells of the rat's insulæ are very similar to those already described for the guinea-pig. Large columnar cells with large nuclei arranged in definite bands consisting of one to two rows of cells occupy the central portion, while at the periphery most of the cells are smaller, the nuclei more crowded and the arrangement less band-like; the blood-vessels are smaller and less prominent. Fig. 3 represents the appearance of a section through the central part of a small area of Langerhans from the rat's pancreas.

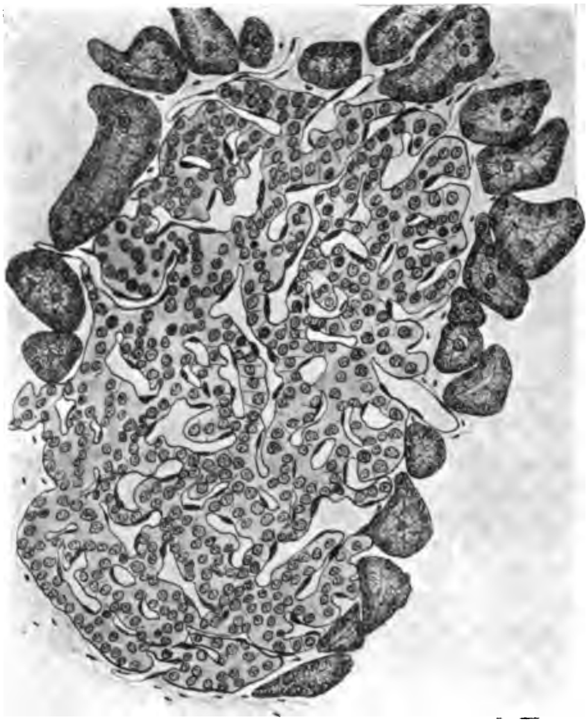


Fig. 3.—Section through central part of area of Langerhans from pancreas of rat. X 200.

In this series of sections, there was passive congestion, so that all veins and venous connections were filled with blood, while the arteries were as a rule empty. It was therefore an especially favorable series of sections for determining the relation of the vascular supply to the pancreatic vessels. As stated before,

v. Ebner and Diamare regarded the vessels of the areas as venous, while Laguesse, Opie, Kühne and Lea, Pensa and others believed it to be arterial, and Hansemann finds only capillary connections. After a most careful investigation of this series of sections as well as of many others from this and many other species, I have never been able to demonstrate an arterial connection with the vessels of the areas, since I have not found a vessel with distinct muscular coat either entering the areas or sending branches into them. Sometimes an arteriole was seen apparently approaching an area, but on tracing it through the series of sections, it was seen to turn aside or pass over the area without communicating with it. In cases of venous congestion, the blood-vessels of the islands are always packed with blood, and in a few cases, as in Fig. 4, I have been able to trace the main vessel, which have regarded as afferent, directly to a vein, beside which ran an artery with distinct muscular coat. In double injections, as will be shown later, it is always the venous injection mass which fills the blood-vessels of the areas. I have therefore regarded the blood-vessels of the areas as venous with abundant capillary connections with the surrounding interacinar capillary plexus.

Nearly always in tracing areas through a series of sections, at least one and sometimes more than one larger vein, usually arising either from an interlobular or intralobular vein, has been seen to enter the area and this I have designated as the principal or afferent vein, while several smaller venules and large numbers of capillaries leave the area in all directions. Since all of these vessels have the same structure, it is difficult, except by the size, to determine which are afferent and which efferent, and we can say only that several larger veins and numerous capillaries form a rich plexus of large, irregular, thin-walled blood-vessels, which, in accordance with Minot's characterization, I regard as sinusoids. The insular vessels always have a different arrangement from that of the interacinar vessels, but the arrangement is variable, especially in sections. Since the areas of the rat are usually much longer than they are wide, the principal vessel usually enters at the side, often passing, as in Fig. 4, to near the center of the island and there breaking up into branches, which divide and redivide, the secondary branches often communicating by means of capillaries. Since Fig. 4 represents a section only 5$\mu$ in thickness, some idea of the rapidity of the branching and the centralization of the larger branches may be gained, although a thicker section would show greater continuity of the vessels. In sections stained with hæmatoxylin and eosin, the endothelial walls of the blood-vessels seem to rest directly upon the cords of epithelial cells constituting the island, and in most places no connective-tissue capsule can be seen. Where the area reaches the periphery of the lobule as seen in Fig. 3 and as is quite common, especially with the larger areas in the central portion of the pancreas, loose connective tissue forms its boundary and occasionally nucleated connective tissue may be seen in other places separating the area from the acini. In sections stained with Mallory's connective-tissue stain, a delicate blue line is seen separating most of the cells; a thin, but nearly if not quite complete, capsule of connective tissue surrounds the area and sends in still more delicate sheaths for the blood-vessels.

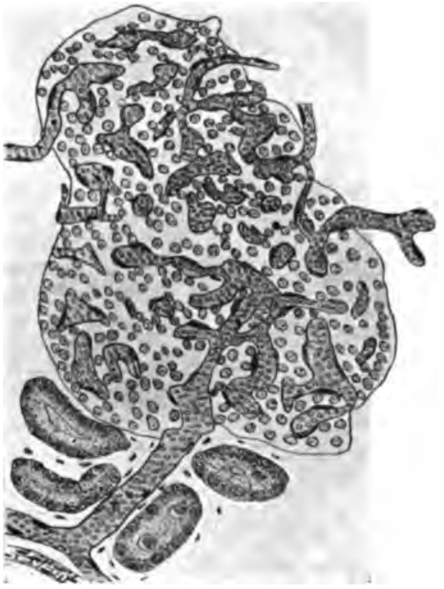


Fig. 4.—Section of area of Langerhans from pancreas of rat in which the veins were filled with blood. The figure shows the connection with the large vein and the arrangement of the sinusoids within the island as seen in a thin section. ×200.

In the *rabbit*, I have found on an average about one area for each square millimeter of section. As the average size of areas measured was .0034 c. mm., about $\frac{3}{1000}$ of the pancreas was insular tissue. The measurements taken were as follows:

| | | |
|---|---|---|
| 1. | 0.12 x 0.22 x 0.09 mm | 0.0024 c. mm. |
| 2. | 0.15 x 0.23 x 0.15 mm | 0.0052 c. mm. |
| 3. | 0.08 x 0.23 x 0.18 mm | 0.0033 c. mm. |
| 4. | 0.12 x 0.18 x 0.10 mm | 0.0021 c. mm. |
| 5. | 0.16 x 0.20 x 0.15 mm | 0.0048 c. mm. |
| 6. | 0.13 x 0.16 x 0.18 mm | 0.0037 c. mm. |
| 7. | 0.15 x 0.23 x 0.08 mm | 0.0028 c. mm. |
| 8. | 0.10 x 0.11 x 0.095 mm | 0.0011 c. mm. |
| 9. | 0.14 x 0.18 x 0.255 mm | 0.0064 c. mm. |
| 10. | 0.15 x 0.15 x 0.10 mm | 0.0023 c. mm. |

A comparison of these measurements shows a considerable difference in the actual size of the areas, since Number 9 is nearly six times as large as Number 8. As may be seen from the measurements, as well as from Plate XII, Figs. 2 and 3, which reproduce two wax reconstructions of fairly typical islands from the pancreas of a rabbit, the islands are nearly always considerably longer in one dimension than in the other two. They are not, however, at all regularly oval. since there are numerous projections which run out between the pancreatic acini. The cells are of about the same types as described for the guinea-pig and rat, but in the sections observed by me there was an unusually large number of the large distinctly contoured eosinophile cells, with large, excentric, deeply staining nuclei. Such cells are more numerous near the periphery of the area. These often seem to merge into the acinal cells so gradually that no line of demarcation can be made out. In some places the lines of nuclei in the bands of cells pass out regularly into the cells of a longitudinally cut tubule which shows the typical basal position of the nuclei and the nuclear stain of the basal protoplasm, eosinophile granules being present in the central portion of the protoplasm. The same or similar appearances are not rarely seen in all forms in which the capsule is very thin and the relation to the acini very close. I have, however, regarded the two structures as separate unless, as frequently happens in the rabbit, the connection can be clearly traced through several sections of the series. This has been done in numerous instances in the rabbit and the connection may be seen in the models represented in Plate XII, Figs. 2 and 3, and also in the section shown in Fig. 5. This appearance can best be explained by the fact shown by Pearce and others that the areas develop from the same epithelial anlage as do the pancreatic tubules, and it may easily be

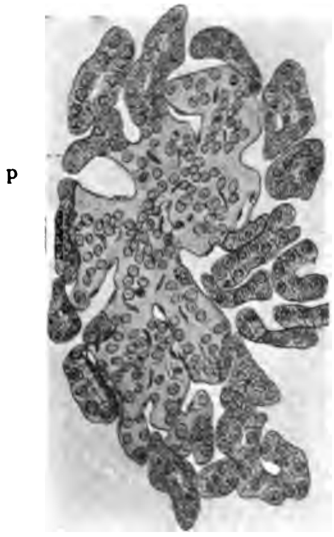


Fig. 5.—Section through the center of an area of Langerhans from pancreas of the rabbit; p, connection with pancreatic tubule. × 200.

that in the rabbit and other lower forms, as well as in some pathologic conditions in the human infant, the connection with the tubules remains unbroken. As seen in Fig. 5, the band-like arrangement of the cells is much less marked in the rabbit than in other species, the bands usually consisting of so many and so irregularly arranged cells that they may be spoken of rather as cell masses.

The character and arrangement of the blood-vessels and connective tissue are very similar to that already described for the guinea-pig and rat. Fig. 6 represents a curious anomaly, two examples of which were found in the sections of the rabbit's pancreas studied by me. These were not reconstructed, but were carefully traced through the entire series of sections in which the area appeared. They were nearly spherical masses of perfectly normal island tissue, which however formed merely a shell, the interior being filled with blood and having an endothelial lining. The occurrence of this blood vesicle or sinus, although only as an anomaly, may serve to lend some support to the idea that the circulation here is sinusoidal. Added interest is given to the anomaly by the fact that Laguesse describes in sheep embryos forms in which the island cells form hollow balls, the interior being filled with blood cells. A similar appearance (blood vesicle) in the petromyzon is mentioned by Pensa.

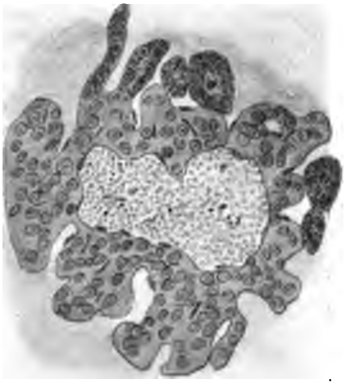
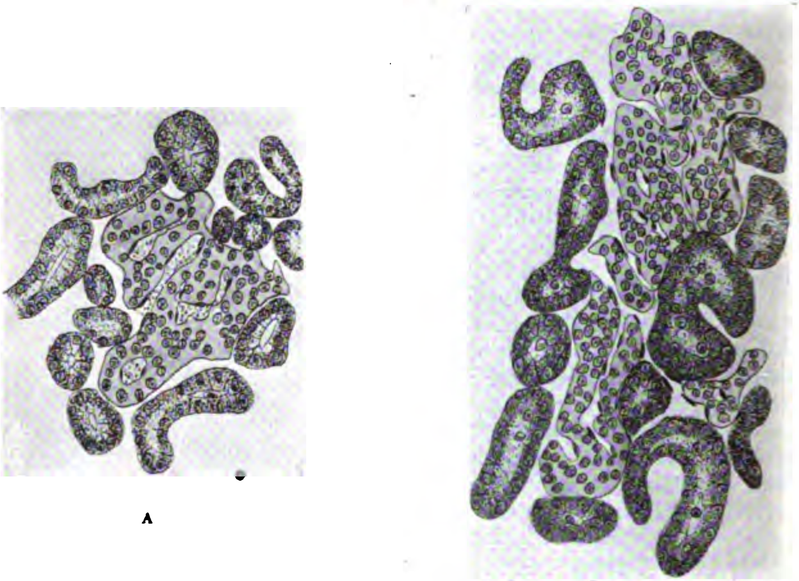


Fig. 6.—Section through center of a vesicular area from pancreas of the rabbit, the interior of the area being filled by a large blood sinus surrounded by endothelial cells which rest directly on a shell of normal island tissue. + 200.

In the *cat*, the areas are as a rule much smaller than in the other forms studied. Twenty areas were measured, taken from different portions of the pancreas and from two different animals. The average size of all the areas was about 0.0027 c. mm. The average number per mm. was 1.08, so that about $\frac{8}{1000}$ of the pancreas consisted of island tissue.

| | Cat I | | Cat II | |
|---|---|---|---|---|
| 1. | .08 x .105 x .18 mm. | .0015 c. mm. | 12 x .16 x .11 mm. | .0021 c. mm |
| 2. | .14 x .19 x .125 mm. | .0033 c. mm. | .11 x .17 x .15 mm. | .0028 c. mm. |
| 3. | .12 x .25 x .10 mm. | .003 c. mm. | .19 x .32 x .135 mm. | .0082 c. mm |
| 4. | 08 x .17 x .09 mm. | .0012 c. mm. | .07 x ,20 x .13 mm. | .0018 c. mm. |
| 5. | .09 x .18 x .14 mm. | .0023 c. mm. | .12 x .19 x .20 mm. | .0046 c. mm. |
| 6. | .11 x .13 x .13 mm. | .0019 c. mm. | .13 x .25 x .125 mm. | .0041 c. mm. |
| 7. | .08 x .14 x .14 mm. | .0016 c. mm. | .11 x .20 x .135 mm. | .003 c. mm. |
| 8. | .11 x .18 x .11 mm. | .0022 c. mm. | .14 x .17 x .12 mm. | .0029 c. mm. |
| 9. | .14 x .16 x .20 mm. | .0045 c. mm. | .20 x .22 x .125 mm. | .0024 c. mm. |
| 10. | .10 x .14 x .15 mm. | .0021 c. mm. | .12 x .17 x .12 mm. | .0028 c. mm. |
| | Average size | .0024 c. mm. | Average size | .0034 c. mm. |

It will be seen that the average size in the second series is greater than in the first and that the areas are generally more nearly spherical or oval than in certain of the other mammals observed, though many of them are considerably elongated, as seen in Fig. 7, B. In Plate XIII, Figs. 1 and 2 are reproduced wax reconstructions of two small areas of Langerhans from the pancreas of an adult cat. These appear, as a rule, unless the sinuses are distended with blood or injected, as nearly solid masses of cells, most of the capillaries being entirely collapsed and seen only as a line of endothelial cells. Especially on the surface are the vascular openings small, so that the cellular elements much predominate. The varieties of cells are in large part the same as those already described; the cells are arranged in irregular masses and to some extent in bands consisting of a single or double row of cells. While the arrangement is variable and all possible forms are seen, it is not unusual to see the cords with the intervening sinusoids passing entirely or nearly across the area, as seen to some extent in both A and B of Fig. 7.



A

B

Fig. 7, A and B. Central sections of two areas of Langerhans from pancreas of cat. × 200.

A number of double injections of the pancreas of the cat were attempted and while the results were not so successful as could be desired, in certain portions of the tissue the injection was such that arteries containing the red granular mass could be found, by the side of which could be traced a vein containing the blue gelatin mass and from there could be traced the principal branches passing to a Langerhans area. Certain of the sections from this tissue were cut relatively thick, so that one section would often contain the whole or nearly the whole of some of the smaller areas, and thus it was possible to get in sections a very good idea of the arrangement of the sinusoids and capillaries in the islands. In Plate XIII, Figs. 3, 4, 5, and 6, are reproduced models reconstructed from thin sections of these injected areas, the blood-vessels only being used in the reconstruction. Three main types of arrangement of vessels may be noted —(1) The vessel runs along the side, either outside or just within the margin, of the area and sends branches mainly from one side into the area; the branches cross the area, branching and winding through it in every direction, and being often connected by anastomosing capillaries. (2) One or two main branches pass through the center sending branches in every direction. (3) The main branch reaches the center of the area and there breaks up into branches which radiate in every direction.

In the models, the arrangement can be quite easily traced in the smaller islands of Langerhans, but in the larger, the arrangement becomes so complicated that tracing is difficult, and the figure can give only a very incomplete idea of the arrangement. In the small islands, there is usually one main (probably afferent) vein, which passes to one of the larger veins, either interlobular or intralobular. Plate XIII, Fig. 3, shows a small and simple area in which the arrangement of the blood-vessels may be easily traced. A relatively large vein runs along the side of the island and sends off a short principal afferent vein. This breaks up almost at once into four smaller vessels, three of which may be seen in the figure. After winding through the area and branching and anastomosing several times, five vessels leave the area, two being capillary in size and the others larger. In Plate XIII, Fig. 4, the main afferent vessel divides, soon after entering the island, into five main branches which branch and anastomose many times, the blood leaving the area by one large efferent vein and six capillaries. The island represented in Plate XIII, Fig. 5, is composed of a plexus consisting of the branchings and anastomoses of two larger vessels and twelve capillaries, while the still larger area represented in Plate XIII, Fig. 6, shows a plexus composed of four larger vessels and fifteen capillaries with their branches. Two of the large vessels form an S-shaped curve and again meet to form the core of the area, the other branches and capillaries twisting around and between them to make up the intricate vascular plexus seen in the figure.

The *human areas of Langerhans* seem much more variable in structure than those found in the other species studied, age and the general condition of the body being more important factors in their structure than appears to be the case in most of the other vertebrates. This is especially true as concerns the insular connective tissue. I have counted and measured areas of Langerhans (1) from a four-year-old child (possibly syphilitic), (2) from a new-born, normal, healthy infant, and (3) from an apparently normal human adult. The measurements are as follows:

| | I | | II | |
|---|---|---|---|---|
| 1. | .21 x .22 x .28 mm. | .0129 c. mm. | .15 x .17 x .13 mm. | .0033 c. mm. |
| 2. | .20 x .28 x .35 mm. | .0196 c. mm. | .20 x .21 x .18 mm. | .0076 c. mm. |
| 3. | .23 x .26 x .30 mm. | .0179 c. mm. | .16 x .18 x .20 mm. | .0058 c. mm. |
| 4. | .16 x .25 x .20 mm. | .008 c. mm. | .15 x .15 x .17 mm. | .0038 c. mm. |
| 5. | .15 x .17 x .23 mm. | .0059 c. mm. | .14 x .15 x .18 mm. | .0037 c. mm. |
| 6. | .23 x .28 x .42 mm. | .027 c. mm. | .13 x .15 x .14 mm. | .0027 c. mm. |

| | | | | |
|---|---|---|---|---|
| 7. | .16 x .225 x .27 mm. | .0097 c. mm. | .16 x .19 x .21 mm. | .0064 c. mm. |
| 8. | .19 x .20 x .265 mm. | .01 c. mm. | .12 x .14 x .17 mm. | .0029 c. mm. |
| 9. | .18 x .22 x .225 mm. | .0089 c. mm. | .17 x .23 x .15 mm. | .0059 c. mm. |
| 10. | .24 x .28 x .29 mm. | .0194 c. mm. | .12 x .21 x .17 mm. | .0043 c. mm. |
| | Average size | .0139 c. mm. | Average size | .0047 c. mm. |

III

| | | |
|---|---|---|
| 1. | .25 x .35 x .325 mm | .0284 c. mm. |
| 2. | .23 x .24 x .225 mm | .0124 c. mm. |
| 3. | .20 x .38 x .225 mm | .0171 c. mm. |
| 4. | .23 x .33 x .225 mm | .017 c. mm. |
| 5. | .18 x .20 x .16 mm | .0057 c. mm |
| 6. | .24 x .25 x .215 mm | .0129 c. mm. |
| 7. | .24 x .30 x .32 mm | .023 c. mm. |
| 8. | .17 x .23 x .275 mm | .0108 c. mm. |
| 9. | .23 x .34 x .25 mm | .0195 c. mm. |
| 10. | .15 x .20 x .215 mm | .0065 c. mm. |
| | Average size | 0.0153 c. mm. |

In Series I of the table, the number of areas averaged 3¼ to each c. mm. of the section, so that about $\frac{4}{100}$ of these sections was area tissue. In Series II, the average number was only 1.3 to each c. mm. of the section and hence only about $\frac{8}{1000}$ of this pancreas was island tissue. The average number of areas in each sq.mm. of the adult pancreas (Series III) is 1.5, making about $\frac{2}{100}$ of the pancreas consist of insular tissue. My figures are slightly different from those given by Opie, Laguesse, and others, since Laguesse finds that on the average only $\frac{1}{100}$ of the human pancreas is island tissue. This can be explained only as an individual variation, since in the figures of the observers mentioned there is quite as marked a variation between any two cases. It is worth noting that the average size in the adult is actually greater than in either of the other cases, while in the four-year-old child it is greater than in the new-born infant. This is quite different from the results frequently reported, since it is usually stated that both the size and the number of islands of Langerhans diminish with age, being greatest in the fœtus and new-born. In the four-year-old child, however, the number in a sq. mm. was greater than in either of the others, and the proportion of area to glandular tissue was much greater in this than in either of the others.

The shape of these areas varies greatly, but is in the main ovoid, though the contour is quite irregular. Something of the shape and general appearance may be seen in Plate XIV, Figs. 1 and 2. Fig. 1 reproduces a wax reconstruction of a fairly typical area from the adult human pancreas, while Fig. 2 is the negative of Fig. I, representing the strands of connective tissue with blood-vessels, etc. Since there was some shrinkage of the epithelial cells, the strands are relatively somewhat larger than they should be, but otherwise indicate the arrangement of the blood-vessels in the larger and more complicated areas like the human. Plate XV, Figs. 1 and 2, reproduce the interior view of the models represented in Plate XIV. The looser and more open appearance of the central portion of the areas is readily seen in these figures. It may also be seen that in some regions two and in others three larger sinusoids run through the area in either direction, so that we find much the same appearance in whatever direction the sections are cut. These large sinusoids are connected at intervals by smaller vessels and, as the greater part of the island is composed of superposed layers of similar arrangement, it may easily be seen why so large a number of sections through the larger portion of the area show a very similar arrangement of cords of cells and blood-vessels. The rich branching of the larger vessels and the connections of the smaller ones may also be seen in the figure.

Since the arrangement of the blood-vessels regulates the arrangement of the bands and groups of cells, a description of the one figure necessarily involves a description of the other. The resemblance of the cords of cells to tubules and acini appears more marked in the interior of the model than in the exterior view. It may be repeated, however, that not only is no lumen found, but there is not even any arrangement of cells which would suggest a lumen. The cells are most irregularly grouped, and the only suggestion of regularity of arrangement seems to be due to their following the arrangement of the blood-vessels. The cells of the human areas vary in size and structure and may be divided into about the same classes as were described for the guinea-pig. The amount and arrangement of the insular connective tissue vary more than any other portion of the structure. In the child's pancreas, there is very little or no connective tissue within the island, the sinusoids resting directly upon the epithelial cells. A space may often be seen between the capillary wall and the epithelial cells, indicating that the cells have shrunken somewhat either in the fixation or as a result of post-mortem change. No such spaces were observed

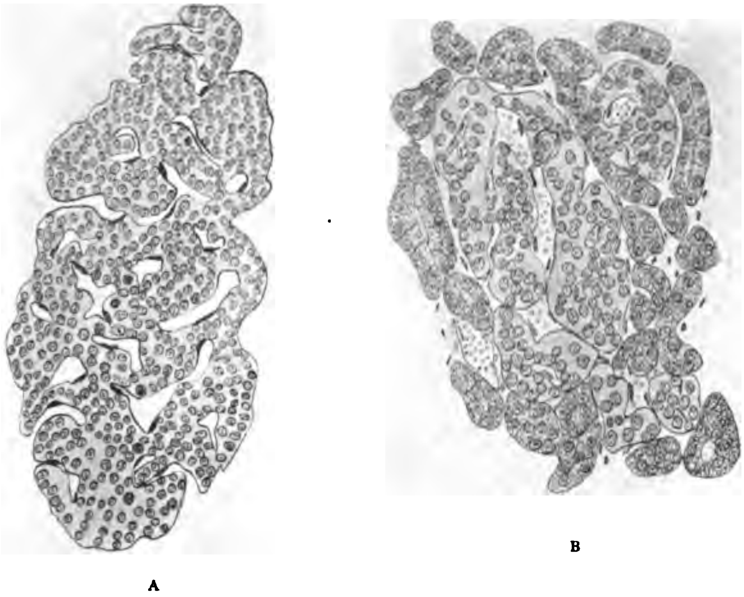


Fig. 8, A and B.—Sections of areas of Langerhans from pancreas of four-year-old child. × 200.

in the fresh pancreas of the new-born infant. No connective tissue can be made out in the pancreas of the four-year-old child with the ordinary stains, but with Mallory's stain a delicate capsule surrounding the area and delicate sheaths around the blood sinusoids can be made out.

In the pancreas of the new-born infant, nearly all the areas observed were found in the interlobular connective tissue and surrounded by it, but no connective tissue could be made out within the areas. In Fig. 8 are reproduced sections of two insulæ from the pancreas of the four-year-old child. The cells are somewhat smaller, the nuclei appearing smaller and more crowded than in the islands of the adult pancreas. This is still more the case in the areas of the younger child. In Fig. 8, B shows an area which has irregular projections into the acinal tissue, as if it had been somewhat broken up by the ingrowth of acini. It is evidently not so near the center of the island as A, which represents a very typical section. In the adult, at least in most of the islands seen by me, there is more connective tissue, forming rather definite trabeculæ, which divide the island into smaller compartments containing the cells; this recalls the description given by Harris and Gow, who, because of this tendency, speak of the human areas of Langerhans as compound. Sometimes the connective tissue forms, with the larger blood-vessels, one or many larger trabeculæ passing through the center, other septa passing to the sides, much as described and figured by Flint. Such an area is shown in Fig. 9, A, showing the central strand cut longitudinally, while in B, it appears to be cut transversely, giving the radiating

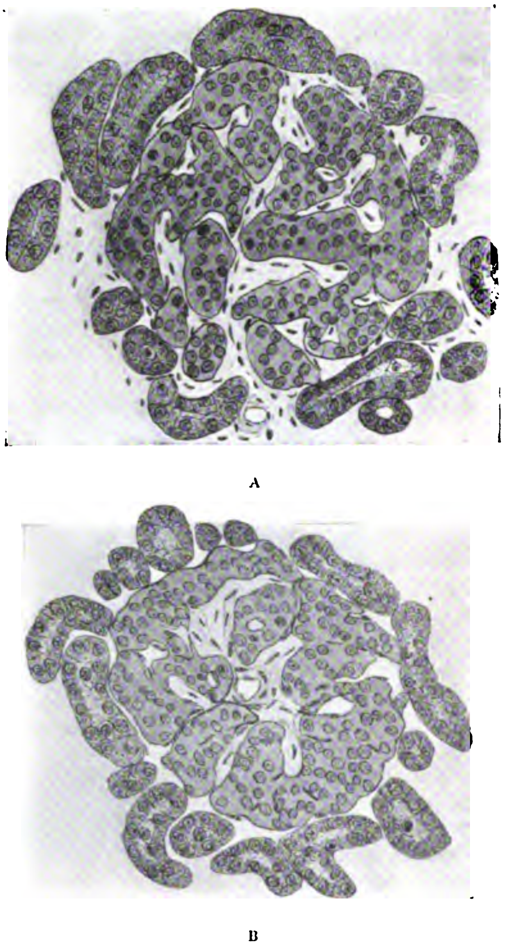


Fig. 9, A and B.—Central sections through two areas of Langerhans of the human pancreas. × 200.

arrangement of the secondary strands, similar to that described for the blood-vessels of the bird. Such differences may be readily understood from a reference to Plate XV, Fig. 2, if we imagine a section cut through the large sinusoids which run nearly parallel to the plane of the section in that figure, we will have much the appearance given in Fig. 9, A. If the section is cut in the same direction, but between two layers of the larger sinusoids, we will get more the appearance given in Fig. 9, B. In general, however, the strands of connective tissue in the human areas, like the sinusoids of other forms, have a very irregular arrangement, scarcely any two islands presenting exactly the same appearance.

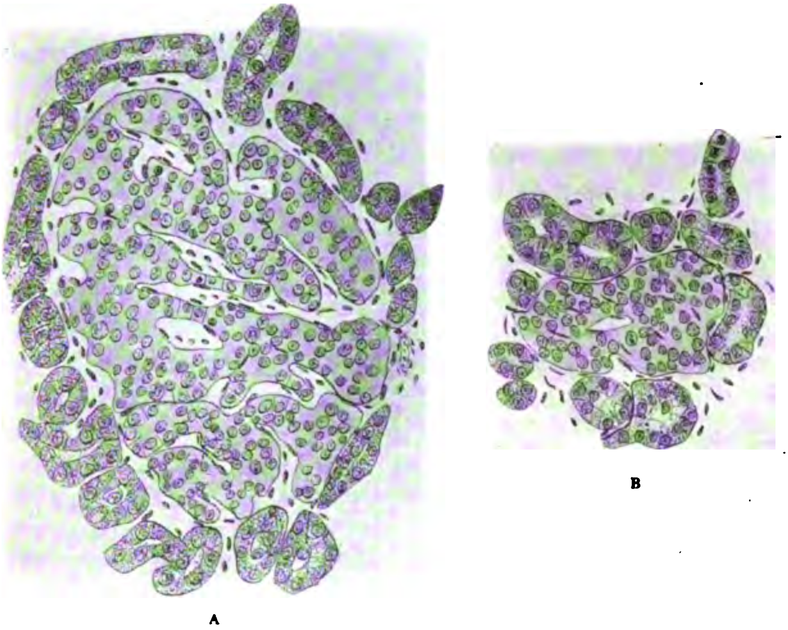


Fig. 10, A and B.—Sections of areas of Langerhans from pancreas of human adult. A, near center of area ; B, near periphery. × 200.

A rather definite capsule of nucleated connective tissue surrounds most of the areas. The only thing which is constantly noted, in the child as well as in the adult, is that in the interior of the islands the strands of connective tissue as well as the blood-vessels contained in them are larger and the masses of cells smaller and more band-like, while the individual cells are generally larger than at the periphery. This makes it easy, regardless of the size of the section, to distinguish a central from a peripheral section. Fig. 10 may serve to illustrate this point, A, of this figure, as well as A and B of Fig. 9; representing sections from near the center of an area, while Fig. 10, B, represents a section near the periphery. The increase of connective tissue with age or with general sclerosis of the gland and the very small amount in younger individuals and in animals seem to indicate that the formation of the connective tissue within the islands is secondary.

In birds, the areas of Langerhans have been found by Mouret, Harris and Gow, Pugnát, Diamare, and others. Diamare asserts that after careful study of numerous serial sections, he is able to affirm that this structure in birds is entirely similar to that in other vertebrates—"the islands consist of very vascular epithelial cords." Pugnát studied the pancreas in many species of birds, but described it especially in the dove. He states that the pancreas consists of three lobes, each with a distinct duct and that the cells do not form acini, but rather a reticular structure like the liver. The islands, he states, are large and irregular, consisting of small, elongated cells with indistinct protoplasm and faintly staining nuclei. Both Mouret and Pugnát regard them as lymphoid structures. I have examined the areas of Langerhans in the dove and in the goose and found them similar, but because of the greater distinctness of the islands in my sections from the dove, my descriptions and figures will be taken from this animal.

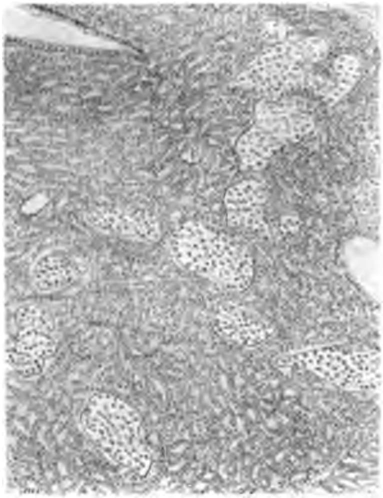


Fig. 11.—Portion of section through central part of dorsal pancreas of dove. × 50.

In the *dove* the two main lobes of the pancreas are found n the loop of the duodenum, one ventral and one dorsal and separated by the mesentery. In the ventral lobe, the areas of Langerhans are few, small and distinct. In the dorsal lobe, on the other hand, they are large and prominent, and it is from this lobe that my figures are taken. The pancreas was congested and all the blood-vessels of the areas were filled with blood and hence were very distinct. It is difficult, on account of the diffuse character of these areas, to determine with any exactness their size or number. They are larger and more numerous near the central part of the lobe than at the periphery. Fig. 11, a low-power drawing of a small portion of this part of the dorsal pancreas, may serve to show the size and irregularity of shape and apparent number of the islands in this region. Several of these, however, unite into one area in a later section, so that the number is smaller and the size greater than is apparent in any one section. The lighter areas represent the islands of Langerhans, and the darker portion the glandular tissue, which I may say in passing seems to me to form definite tubules and acini, as in the pancreas of other vertebrates and not, as stated by Pugnát, a recticular stucture like the liver, although under low power it does present a reticular appearance.

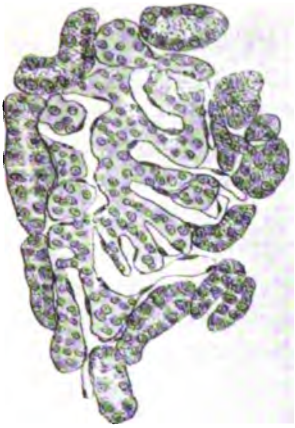


Fig. 12.—Section through central portion of dorsal pancreas of dove. × 200.

The cells of the bird's islands are smaller and more uniform in size and structure than those found in the islands of any other vertebrate examined, scarcely any variation in size or stain of nuclei being noted. The cell outlines can scarcely be made out. The nuclei are small, round, or more often oval, with a very distinct nucleolus. Both nucleus and protoplasm stain faintly. The cells are arranged in definite cords between the sinusoids, which here are wide and prominent because of the congestion. Fig. 12 reproduces a central section of an area of Langerhans from the pancreas of the dove, the blood cells in the sinusoids being omitted. Fig. 13 reproduces three areas of Langerhans from the pancreas of the dove, in which the blood-filled sinusoids are especially emphasized, the cells appearing lighter in the figure. They show three types of arrangement: A shows the origin from a larger vein of the main afferent vessel, which enters the island, passing along one side and dividing into two main branches near the end. Most of the other branches are given off from one side of the main sinusoid, these again dividing and passing in different directions. A large branch is given off from the main sinusoid, which passes back, winding partly under the latter, and supplies the proximal portion of the area This is

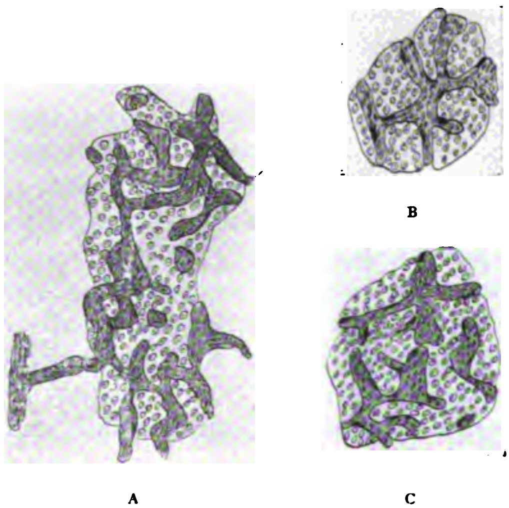


Fig. 13, A, B, C.—Three sections of physiologically injected areas of Langerhans of dove, the injected sinusoids being especially emphasized in the figure. × 200.

a relatively thin section, so that many of the tortuous capillaries are discontinuous, but it indicates very well the connections and arrangement of the insular vessels. B shows the main sinusoid passing through the center of the area and sending branches to either side, while C represents a radial branching from a central large sinusoid. C might, as will be readily seen, represent a transverse section of B.

In the frog, the islands stain more deeply and are so diffusely and irregularly scattered among the pancreatic tubules that counting and measuring are very difficult. It is almost impossible to determine how many of the small island masses seen among the acini belong to a single area or to be certain as to the limits of the areas. The most of the area sections seen by me were relatively small, averaging .002 c. mm. in size, the dimensions averaging about 90 μ x 120 μ x 210 μ. The cells differ somewhat in character from those in the other areas studied, since very tall columnar cells with elongated, deeply stained nuclei predominate. These are crowded together, usually in a single row, especially in the central portion to form the very distinct cell bands so characteristic for amphibia; they resemble those described and figured by Diamare for the triton. He states that "the small epithelial cords are rather delicate, formed of rather slender cells, closely approximated and are separated by large capillaries. These last evidently correspond to the 'large venous sinuses' of v. Ebner. No capsule separates the areas from the zymogenic tissue." Pensa also in speaking of the cylindric cells of the islands of amphibia, says: "These cells are not aggregated into cords but are arranged in linear series, one after the other in single file." In addition to these narrow crowded cells which make up the central cords, masses of polygonal cells with spherical nuclei are usually found, especially at the periphery of the islands. V. Ebner described these islands in the frog as early as 1872, stating only that they showed no lumen penetrable by a mass injected into the duct and that rather large veins are found near the islands. In his later communication in 1899 he states that he regards the vessels of the areas of Langerhans in the frog as venous.

Fig. 14 represents a very typical area from the pancreas of the *frog*, with its tall, narrow columnar cells with oblong, deeply stained nuclei arranged in rather regular bands. Polyhedral cells with round or oval nuclei staining more faintly are scattered irregularly between the columnar cells and form more definite masses at the periphery. The sinusoids are distinct with simple endothelial lining and no connective tissue demonstrable, either surrounding the area or around the intrainsular sinusoids.

I have made no study of fish and reptiles, but Diamare and Harris and Gow and others have described large insulæ in reptilia, and Diamare and Rennie and others have found similar structures in fishes, the islands in these forms having the same structure as in the types studied by me; certain of the very large ones are, however, constant in position and, in some species of fish, are independent of the glandular tissue of the pancreas.

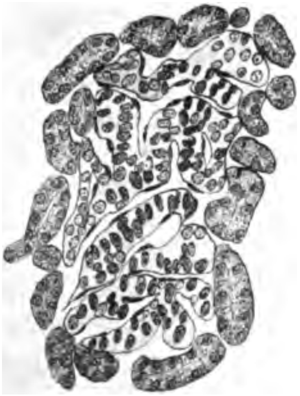


Fig. 14.—Section of area of Langerhans from pancreas of frog. × 200.

In general, then, it may be said that areas of Langerhans of very similar structure are found in all species of vertebrates that have been examined. In all species, they consist of cords or masses of epithelial cells derived from the same anlage as the pancreatic acini and sometimes retaining their connection with the glandular tubules. The cells vary somewhat in type in any one area, but the same or similar types are found in all the species examined, except the frog and bird; in the bird the cells are uniformly small and generally oblong, both nucleus and protoplasm staining poorly; in the frog, the predominant cells are tall and columnar with long deeply stained nuclei, the cells being packed together in single rows separated by blood-vessels. In all cases, the cords of cells are separated by large, irregular, anastomosing blood-vessels, having a complete endothelial wall and no or very little adventitia. In most forms, the endothelium seems to rest directly on the epithelial cells, and the connective tissue, when present, appears to be a secondary development, as in the somewhat sclerotic, adult human pancreas. The vessels correspond to Minot's definition of sinusoids. The vascular network of the islands is made up of the windings, branchings, and anastomoses of several larger vessels of venous origin and large numbers of capillaries, which communicate intimately with the interacinar capillary plexus. The largest sinusoids are found in the central part of the islands, where the cords of cells are relatively small. The periphery of the areas is generally much less vascular. The largest areas found in my series were those of the rat, those of the guinea-pig being second. The reconstructions show that the cords of cells have the external form of branching and anastomosing tubules with occasional alveolus-like enlargements ; they are, however, solid structures with no lumen and no arrangement of the cells and nuclei which would suggest a lumen.

The areas are generally larger and more numerous in the central portion of the pancreas, especially in the bird, guinea-pig, and rat. In most species, the islands are intimately related to the glandular tissue, the capsule, when present, being so thin and delicate that it is demonstrated with difficulty. In the newborn child, however, most of the areas lie in the interlobular connective tissue and are surrounded by it; in the guinea-pig, the largest areas are in the connective tissue surrounding the larger ducts and are independent or practically independent of the pancreatic acini; and in some species of fish certain of the islands are very large, constant in location, and entirely independent of pancreatic tissue. In the human accessory pancreas, islands may or may not be present, the number reported in which they were absent being approximately equal to that in which they were present.

It is very natural to inquire concerning the function and meaning of an organ so constant in its presence and structure at all ages and in all species, occurring so early in embryonic life, and maintaining its vitality with so few changes through all conditions, and it is this side of the question which has interested the great majority of the investigators of the islands of Langerhans. The most varied views prevail. The discoverer, Langerhans, suggested the possibility of a relation to the nervous system, since these structures are often found in close proximity to the sympathetic ganglia and the related nerves and staining reaction of their cells in ordinary dyes is not dissimilar. Because they are found in embryonic life and appear larger and more numerous in the embryo and new-born than in the adult, Gibbes, Harris and Gow, and others, regarded them as embryonal remains. Krause, Kühne and Lea, Renaut, Sokoloff, Dieckhoff, Pischinger, Pugnát, Mouret, and others basing their theory on the character of the cells, regarded them as lymphoid structures. Dogiel and Tschassownikow considered them as exhausted acini, or as Dogiel states "todte Punkte" because he found no connection with the duct and found fat globules in the cells.

Lewaschew, Laguesse, Mankowski, Statkewitsch, Pischinger, Saviotti, Kollossow, and many others assert that the islands are merely temporarily changed acini, which may change back into pancreatic acini. The reasons for this view are well expressed by Mankowski. He states that the number of islands increases during the period of gland activity and diminishes during rest; that the islands .of Langerhans represent one of the morphological stages of activity of the pancreas. Every lobule of the pancreas must, at the end of its period of active secretion, pass into the "Stadium der Langerhans'schen Inseln," which represents the morphological phenomena of the greatest exhaustion or the greatest activity. He also notes the occurrence in any one section of various stages of transition between gland acini and the insulæ. Finally Zunz, Ssobolew, von Ebner, Schultze, Jarotzky, Sauerbeck, Diamare, and probably the great majority of the very recent writers on the subject regard the islands as independent organs, vascular glands, arising from the same anlage as the pancreas, but having a different function,—the elaboration of a secretion which is poured into the blood. This theory is based partly upon clinical and partly upon experimental data and will be considered more in detail later.

If perfectly fresh and well-fixed pancreas is examined, the cells of the islands, with their relatively small nuclei and prominent protoplasm and their arrangement into definite bands separated by sinusoids, are sharply differentiated from adenoid tissue, which can sometimes be seen in close proximity. The development of the areas from an epithelial anlage also speaks against the theory of the lymphatic structure. That they are not embryonal remains may be readily seen from the fact that they do not in any sense degenerate in adult life, but remain alive, with a rich blood supply, and are, according to my experience, only relatively larger and more numerous in the young than in the adult. The rich blood supply, the absence of any appearance of degeneration, and the occurrence of dividing cells, as noted by Bizzozero and Vassale, by Schulze, and as I myself have seen, all refute the theory that they are pancreatic acini undergoing regressive changes. These dividing cells were especially numerous in the islands of the young child's pancreas, but some cells undergoing division were seen in nearly every section stained in Heidenhain's iron-lac-hæmatoxylin. The theory that the areas are being constantly derived from pancreatic acini and changing again into the glandular tissue is based (1), on the increase or diminution in the number and size of the areas during different conditions of digestion, and (2), on the occurrence of transitional forms, especially as shown by Lewaschew, after repeated pilocarpinization. Opie and Hansemann have repeated Lewaschew's experiments with pilocarpin, making careful counts of the islands, but have found no increase in the number of islands and no transitional forms. Ssobolew tested hunger, active digestion, and pilocarpin and found areas only slightly altered in number.

There is no question that structures do appear at times which might be interpreted as transitional forms. I have not infrequently seen groups of tubules in which the characteristic differentiation into two zones was entirely absent, the nuclei being centrally placed in the cells; these groups looked not unlike large islands of Langerhans, especially if, as sometimes happens, no lumen could be made out in the tubules. Sometimes, also, the eosinophile cells of the areas may be so numerous and so arranged as to resemble somewhat the tubules just mentioned. In the guinea-pig, I have once or twice seen, in apparent connection with one of the central areas, tubules having no outer basophile zone and having the nuclei centrally placed. These, however, were very rare, and by tracing them through the series of sections, I was generally able to convince myself that they were merely contiguous with the areas and not continuous. The appearance of the pancreatic tubules varies so much under different conditions, and the relation of the tubules to the islands is, in most animals, so intimate, that it would not be difficult in almost any section to find structures which might be regarded as transitional. I have seen none, however, which could not be explained in some other and more rational way.

As to the size and number of the islands in different stages of digestion and under different diet conditions, Mankowski says that the areas increase during digestion, while Hansemann states that the apparent increase is due only to a more marked differentiation because of the change in the acini themselves.

To test the *changes produced in the islands of Langerhans by digestion and diet*, I have examined, measured, and counted a large number of islands from a considerable number of guinea-pigs under the following conditions: (1) normal in full digestion; (2) after different periods of time without food or drink; (3) after they had been kept on pure carbohydrate diet for different periods; (4) after the same periods on pure meat diet. As the guinea-pigs had to be forced to the meat diet, the earlier animals showed purely the changes of inanition, and I have not included them in my table. In order to make the conditions as nearly parallel as possible, I, in all cases except the first, killed the animal about fourteen hours after eating, divided the fresh pancreas into three approximately equal portions, fixed them in saturated bichloride solution; and cut the sections longitudinally in the direction of the main duct and through that portion of the gland which included the main duct. The sections therefore represented approximately the same regions of all the organs examined. I have measured sections of areas rather than the three dimensions, since the work previously reported has been based on the appearances noted in sections. The variations in size due to cutting through peripheral portions of the islands were overcome by measuring large numbers and taking the average. I counted and measured all the islands in the entire section in each case and in at least five sections, so that my average may certainly represent a true average for the areas in this region of the pancreas. While some qualitative changes were noted in the areas,—such as an increase or diminution of the number of eosinophile cells, a granular change in the cells, atrophy of cells with increase of intercellular substance,—there were none which could be regarded as constant for any one experiment and constantly increasing with the duration of the experiment. The table given below gives a brief synopsis of the main data gained from the counts and measurements. The largest number of very large islands was seen in the animal which had been kept on a meat diet for sixty days, while the proportion of island tissue to pancreatic glandular tissue is constantly greater under meat diet than under carbohydrate diet or in hunger. But since, as may be seen from the measurements given of the normal areas, there are marked individual differences even under the same circumstances, I believe that this variation is due to individual peculiarities, although Ssobolew states that the size and number of the areas are quite constant for each species. In the following table, 1, in each case, represents the splenic third of the pancreas, 2, the middle third, and 3, the end nearest the duodenum.

| Experiment. | Av. no. per mm. | Av. size in sq. mm. | Proportion of gland to island. | Size of largest area in sq. mm. | Size of smallest area in sq. mm. |
|---|---|---|---|---|---|
| Normal (full digestion) | | | | | |
| 1. | .35 | .02 | 1: .007 | .31 x .38 | .04 x .06 |
| 2. | .42 | .014 | 1: .006 | .20 x .21 | .04 x .06 |
| 3 | .68 | .021 | 1: .014 | .21 x .43 | .05 x .06 |
| Hunger–36 hours | | | | | |
| 1. | 1.54 | .012 | 1: .018 | .13 x .37 | .04 x .05 |
| 2. | 1.22 | .0064 | 1: .008 | .19 x .20 | .03 x .04 |
| 3. | .71 | .014 | 1: .01 | .24 x .36 | .03 x .05 |
| Hunger–46 hours | | | | | |
| 1. | .346 | .023 | 1: .008 | .40 x .54 | .02 x .04 |
| 2. | .4 | .014 | 1: .015 | .15 x .20 | .03 x 11 |
| 3. | .18 | .036 | 1: .006 | .25 x .48 | .05 x .06 |
| Hunger–52 hours | | | | | |
| 1. | .46 | .018 | 1: .008 | .19 x .52 | .02 x .04 |
| 2. | .52 | .015 | 1: .008 | .19 x .23 | .05 x .06 |
| 3. | .51 | .009 | 1: .005 | . 3 x .18 | .03 x .04 |
| Carbohydrate diet 30 days | | | | | |
| 1. | .39 | .018 | 1: .006 | .18 x .33 | .0 x .08 |
| 2. | .74 | .013 | 1: .009 | .38 x .40 | .04 x .05 |
| 3. | .43 | .019 | 1: .008 | .20 x .35 | .06 x .07 |
| Carbohydrate diet 60 days | | | | | |
| 1. | .39 | .016 | 1: .006 | .19 x .21 | .06 x .08 |
| 2. | .5 | .019 | 1: .0099 | .22 x .34 | .05 x .06 |
| 3. | .52 | .019 | 1: .0098 | .18 x .50 | .06 x .07 |
| Meat diet, 30 days | | | | | |
| 1. | .75 | .015 | 1: .011 | .13 x .50 | .03 x .05 |
| 2. | .69 | .015 | 1: .0102 | .26 x .40 | .05 x .06 |
| 3. | 1.01 | .012 | 1: .012 | .17 x .19 | .04 x .05 |
| Meat diet, 60 days | | | | | |
| 1. | .57 | .032 | 1: .018 | .30 x .68 | .05 x .07 |
| 2. | .54 | .025 | 1: .013 | .27 x .42 | .05 x .07 |
| 3. | .4 | .031 | 1: .012 | .22 x .68 | .06 x .08 |

The so-called transition forms are merely resting pancreatic tubules. The size and number of the islands have individual variations under normal conditions fully as great as occur under varied conditions of digestion, hunger, and diet, and no changes occur under these conditions as has been shown by Opie, Schulze, Diamare, Jarotzky, and by myself. That the protoplasm of the insular cells, as Mankowski himself states, is chemically different from that of the pancreatic cells is shown by the marked differences in their affinity for stains, especially for silver nitrate and safranin. As has been shown by many experimenters, the islands may be well preserved when the gland tissue is extremely atrophied, so that they do not seem to be subject to the same conditions as pancreatic cells. Of especial importance is the fact that islands have been found in some species independent or practically independent of the pancreas. For these reasons we certainly seem justified in the conclusion that the islands of Langerhans are independent organs with an independent function.

For over a century, cases of diabetes have been reported in which the pancreas was diseased, and the tendency has been constantly increasing to regard pancreatic disease as one, at least, of the causes of diabetes. This tendency has been greatly augmented since 1889, when Minkowski showed that in dogs the removal of the pancreas gave rise to glycosuria and most of the symptoms accompanying diabetes in man. Since then, medical literature has abounded with clinical and pathological reports as well as with chemical and experimental studies on the relation between the pancreas and sugar metabolism. After lesions of the islands of Langerhans in association with diabetes mellitus were described by Opie in 1900, discussion has been limited to the relation between sugar metabolism and the islands of Langerhans. I do not intend to enter in detail into the pathological reports, since my own work does not to any extent deal with the clinical side of the question and since pathologists are still at variance in their conclusions. Suffice it to say that Opie, Weichselbaum and Stangl, Wright and Joslin, Herzog, Ssobolew, and many others believe themselves justified in accepting the theory that pancreatic diabetes is due to some disturbance of the function of the areas of Langerhans, while Hansemann, Gutmann, Reitmann, Karakaschew, Herxheimer, Dieckhoff, Benda, and others contend that this theory does not explain and can not be based upon the facts observed.

Recently Sauerbeck has collected from the literature most of the published cases of diabetes in which the condition of the islands was reported and has added to them a number of his own cases. He states that 117 of the 157 cases of the series show abnormal islands of Langerhans. In seven cases the islands could not be found. In one, they were represented by scar tissue. The changes described are as follows: (1) a diminution in size and number; (2) qualitative changes, such as hæmorrhage, fatty degeneration, acute and chronic inflammation, simple atrophy, hydropic degeneration (Weichselbaum and Stangl), sclerosis, and hyaline degeneration. If we reject as too indeterminate the purely quantitative changes, we still have 98 of the 157 cases in which qualitative changes were observed. Most of these changes have, however, in greater or less degree, been reported in non-diabetic cases, and eleven cases have been described by Dieckhoff, Hansemann, Litten, and Ziehl, in which the pancreas was entirely destroyed by suppuration or by carcinoma and yet no diabetes occurred, while non-diabetic cases have been observed in which no normal islands were found. Whether it be that in the complexity of function of the human organs, some other organ or tissue takes up the work of the areas under certain conditions, whereas in other cases they fail to do so, cannot be stated with any certainty. Several writers have asserted an interrelation of function between the pancreas, the liver, and the spleen, while Lorand has recently asserted that he has proved by experiments on dogs a relation between the function of the areas of Langerhans and the thyroid. He therefore states that the areas of Langerhans secrete a substance which neutralizes the poison produced by the thyroid and that diabetes may result either from the increased functional activity of the thyroid or from diminished activity of the areas of Langerhans. However this may be, all that can be said in the present condition of our knowledge is that the anatomo-pathologic investigation of this question has not as yet led to any satisfactory solution.

The announcement of the relation of the pancreas to sugar metabolism gave an impetus to an investigation of this question from the chemical point of view. Arnheim and Rosenbaum asserted the presence of a glycolytic substance in pancreas, in muscle, and in liver, the glycolysis being much increased if pancreatic extract were mixed with either the muscle or the liver extract. Šimáček claims to have isolated from the pancreas by precipitation with alcohol and ether a substance which, under anaërobic and aseptic conditions, causes an energetic alcoholic fermentation. Feinschmidt also obtains a glycolytic substance from liver, muscle, and pancreas, while Stoklasa has found a similar substance in plants and in numerous organs and fluids of the animal body, and Croftan claims to have found a glycolytic substance which he calls trypsin in the human blood. Stoklasa, Šimáček, and Feinschmidt find as the products of the glycolysis alcohol, carbon-dioxide, and acids. Cohnheim, however, claiming that this alcoholic fermentation is the result of bacterial influence, has published a series of articles in which he shows that there is present in muscle a glycolytic ferment, which, however, is inactive until acted upon by a substance in the pancreas which he calls an "activator." This substance is not destroyed by boiling and is soluble in water and alcohol but insoluble in ether. Increasing the amount of the pancreatic extract or of the ether precipitate of the same increases the influence up to a certain point, beyond which any further increase diminishes the glycolytic action. He regards this action as analogous to that of Ehrlich's amboceptor and complement. The presence of blood in the muscle causes glycolysis without addition of pancreatic extract and sometimes the glycolysis is diminished when pancreas is added. This fact would indicate the presence in the blood of variable amounts of the activator principle of the pancreas. He concludes that this substance is not a ferment since it is not destroyed by boiling, but is analogous to the other products of internal secretion, adrenalin, iodothyrin, and secretin. Fichera examined liver, muscle, cartilage, and epithelium of depancreatized dogs and found that the glycogen diminished and finally disappeared in about thirty days after the operation and concludes that the main phenomena of diabetes are a diminution of normal amylogenesis and a weakening and often disappearance of the glycolytic functions of the organs.

As has already been mentioned, in 1889 Minkowski succeeded in totally extirpating the pancreas of dogs, the operation being followed by glycosuria and other symptoms of diabetes. Since then the operation has been repeated on dogs, cats, rabbits, birds, frogs, turtles, and eels, by numerous investigators, among whom may be mentioned de Dominicis, Minkowski, Harley, Kausch, Weintraud, Aldehoff, and Marcusi. While the results obtained by different investigators have varied somewhat, owing no doubt to the different methods used in the operation and also in the subsequent urinalysis, glycosuria following pancreas extirpation has been found in all the species examined, with the exception of ducks and geese, in which Kausch was able to demonstrate a hyperglycæmia but no glycosuria. De Dominicis found glycosuria in only about two thirds of the seventy animals (dogs, cats, rabbits, pigs, etc.) upon which he operated, but in all there were some changes such as polyuria, polyphagia, polydipsia, azoturia, phosphaturia, etc. Glycosuria was affected somewhat by diet, drugs, etc. Lüthje and others assert that the removal of the pancreas does not entirely destroy the function of glycolysis, since they found that the sugar disappeared from the urine while it was still present in the blood. Lépine and Thiroloix state that diabetes does not follow extirpation of the pancreas, if the animal is starved for some days before the operation. Pflüger, in order to test the constancy and permanency of glycosuria, removed the pancreas in a considerable number of animals, being careful that the extirpation should be total. He found that in every case glycosuria resulted and led to the death of the animal. He noted also that the liver always increased in size with an increase in fat, but that glycogen was absent.

After partial extirpation of the pancreas, the results are more variable and depend, as Minkowski thought, not so much on the size of the portion of the pancreas that is left as on its condition. The same seems to be true in most cases whether the remnant is left in situ or is transplanted under the skin, provided the blood supply is not interfered with.

In 1891, Vassale first noted the preservation of the islands and the glandular atrophy resulting from ligation and section of the pancreatic duct in rabbits. In 1898, Katz and Winkler found the islands of Langerhans preserved for some time after the ligation of the duct. In 1900, Schulze tied off small portions of the pancreas in guinea-pigs, causing a complete atrophy of the ligated glandular portion, which was replaced by connective tissue, while the islands were unaffected. After eighty days he finds mainly connective tissue with a few dilated ducts and the normal areas of Langerhans. Since no glycosuria resulted from his experiments, he drew the conclusion that the areas of Langerhans are vascular glands of the type of the hypophysis, having an internal secretion whose function is probably to regulate the sugar content of the blood. In 1901, Mankowski repeated Schulze's experiment, tying two ligatures, and examining the portion between the ligatures, as well as the portion on either side of the ligatures. His results and conclusions were directly opposed to those of Schulze. He found both areas of Langerhans and pancreatic tubules atrophic and sclerosed as a result of his operation; as he notes an increase in the number of islands during digestion and a diminution in the resting stage of the pancreas, he decides that the islands are merely temporarily changed acini.

In 1902, Ssobolew ligated the duct and divided it between the two ligatures in dogs, cats, and rabbits. In cats, he found that the ducts generally reunited and became permeable before any extreme atrophy could take place. He found rabbits most favorable, since the duct could be tied and cut without injury to the pancreas. In the rabbit's pancreas, he found, as did Schulze in the guinea-pig, that the gland tissue became atrophic while the areas were well preserved up to the four hundredth day, at which time his observations ceased. At that time, the gland consisted only of the normal areas, surrounded by connective tissue containing the main duct. He describes the following changes in the glandular tissue: (1) There is loss of zymogen granules, protoplasm is homogeneous, and nuclei are irregular, shrivelled, and deeply stained. (2) Cells disappear and membrana propria collapses. (3) Centro-acinar cells are not seen after the eleventh day. (4) The changes in the glandular cells consist of atrophy, vacuolization, granular change, and sometimes indirect cell division. (5) Cells of small ducts increase and to some extent also those of the medium-sized ducts. (6) Between the fourth and fifteenth days, there are mucous degeneration of cells of large ducts and thickening of walls. (7) After the thirtieth day there are atrophy of small ducts, collapse of membrane, and degeneration of epithelium. (8) New formation of connective tissue is interlobular, and there is much new elastic tissue around ducts and islands. (9) The number of blood-vessels diminishes after twenty days, the number of nerves after from sixty to seventy days; the number of ganglia and of cells in the remaining ganglia diminish and Vater-Pacinian bodies degenerate and do not reappear. (10) If measures were not taken to prevent it, there was a new formation of excretory ducts through which the secretion could be carried to the duodenum. Pawlow and Smirnow and Tiberti also note this regeneration of the duct and with it of the gland tissue, if the degeneration has not gone too far. (11) As to the areas, Ssobolew makes an important statement. While he finds the areas normal in the rabbit four hundred days after the operation, he finds that earlier, between the thirtieth and one hundred and twentieth day, some of the islands degenerate and many are reduced in size, but that there are individual differences in the power of resistance.

In none of the animals operated upon, did Ssobolew find glycosuria, but he does not state how frequently he examined the urine or how long after the operation he continued to do so. The point is of especial importance since Hédon in 1894 noted a slight transient glycosuria from the first to the fourth month after the pancreatic duct was filled with oil, thus causing atrophy of the gland. Sauerbeck (1904) calls attention to the same fact and also to its significance. He states that in rabbits (the same species on which both Hédon and Ssobolew operated) he noticed after ligation of the duct a slight glycosuria developing after the first month and at the same time a distinct alteration of the islands. This coincidence of facts is extremely suggestive for the island theory of diabetes.

Zunz (1905) after ligation of the excretory duct of the dog's pancreas noted the same atrophy of the gland parenchyma with preservation of the areas as described by Schulze, Ssobolew, and

Sauerbeck. Hansemann (1902), however, after ligating the duct in ten dogs, states that the atrophy extends only a short distance beyond the ligature and that the gland is later restored to a considerable extent. The islands may be well preserved or they may undergo fibrous degeneration. Lombroso (1905) tied and cut the duct in dogs and pigeons and found the glandular parenchyma practically unchanged. He states also that the islands show changes proportionate to those in the glandular tissue. It would seem to me probable that the inconsistent results obtained by these two investigators are due either to incomplete obstruction of the duct or to the regeneration of the duct which is so commonly met with, since their operations seem to have had so little effect on either the structure or the function of the pancreas. Lépine (1905) comes to the conclusion that the acini, as well as the areas of Langerhans, take part in furnishing the internal secretion to the blood and bases his conclusion on the following facts: (1) in some diabetics the insulæ are affected and in others, the glandular tissue; (2) after ligation of the pancreatic duct or injection of oil into it, the glycolytic power of the blood is greatly increased. As interference with the outflow of the secretion can affect only the gland parenchyma and not the islands, he considers this as sufficient proof that the acini as well as the islands form the glycolytic principle which is given to the blood.

Since the experiments of Schulze and Ssobolew and others showed that by ligation of the duct and interference with the outflow of the pancreatic secretion, a complete atrophy of the glandular acini of the pancreas was caused, while the areas of Langerhans remained unchanged, it seemed possible that by their method the areas of Langerhans might be isolated in considerable portions of the pancreas and the physiologic action of their extract determined; it was with this purpose that my experiments were undertaken. My experiments were performed in great part upon cats. Three were attempted upon guinea-pigs, but these were unsuccessful, as the pigs died during the first three days after the operation. In order to obtain as large an amount of extract as was consistent with the life and well-being of the operated animal, I endeavored to pass my ligature as close as possible to the point of union of the two ducts. This point varies somewhat in different individuals, but in the main is about as represented in Fig. 15. In this figure, the ducts had been exposed by dissection throughout their entire length, having been first filled with a blue solution. The blood-vessels had been previously injected with a red mass. The two halves of the pancreas were then laid back to expose the duct. The delineator has not represented quite accurately the relative size and length of the two portions of the gland, but the point of junction of the ducts and the relation of the ducts to the blood-vessels at the point of operation are nearly correct. My ligature, then, in five operations was at *t;* five glands were ligatured at *s* and *t*, and two at *s* only; ten had two ligatures tied tightly in the neighborhood of *s*, the pancreas being completely divided between the ligatures and the severed ends thoroughly cauterized to prevent the re-

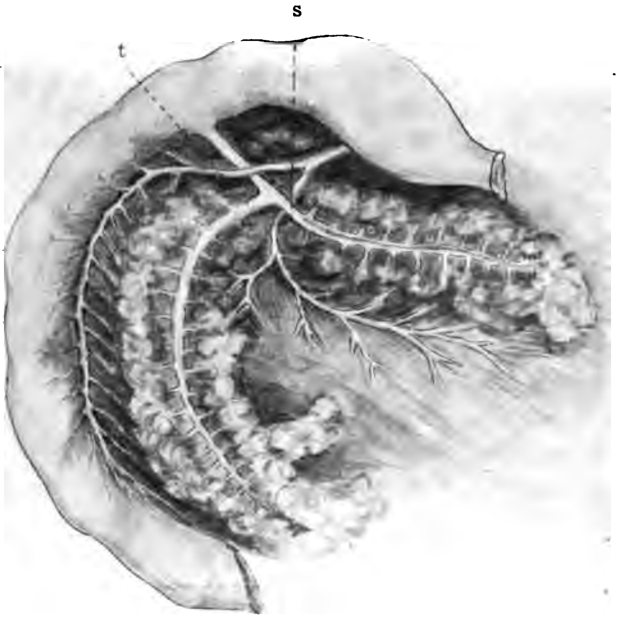


Fig. 15. Pancreas of cat laid open to show main ducts and their relation to the large vein near the junction of the ducts. The letters are explained in the text.

union of the duct which had apparently taken place when the gland had been simply ligatured. In seven cases, after dividing the gland between two ligatures at *s*, a fold of omentum was drawn between the two ends and sewed or tied in place.

The animals lived after the operation from eighteen hours to 197 days. Ten of them, on account of an acute inflammation (three cases) or inanition (the cats often refused all food for the first week or ten days after the operation), died too early to hope for a sufficient degeneration of the gland tissue to attain even an approximate isolation of the islands. The changes found in the glandular parenchyma were much the same as those described by Ssobolew. The larger ducts were much dilated and filled with desquamated epithelial cells and detritus. The smaller ducts and the acini could scarcely be differentiated, since the gland cells had in the main early lost their differential staining power, the outer zone no longer taking the basic stain nor the inner the acid stain, but both staining alike. The cells in both small ducts and acini in many cases disappeared. The membranæ propriæ then collapsed and appeared like strands of connective tissue. The interlobular connective tissue was greatly increased, so that the lobules were much compressed, but still retained the appearance of lobules. In few, if any, of my cases has the lobule been entirely replaced by connective tissue except in the immediate neighborhood of the ligature, where often both gland tissue and areas of Langerhans had completely disappeared to give place to new connective tissue. This was especially the case in those animals in which the gland was divided and cauterized.

In my earlier operations, no special effort was made to avoid including the blood-vessel in the ligature and in some of these cases islands as well as gland tissue had suffered atrophy. In the other experiments, in which an effort was made to avoid interference with the blood supply of the gland, the areas were quite well preserved regardless of the extent of atrophy of the gland tissue. In those animals killed during the first six weeks or two months, however, many of the islands appeared smaller and less numerous than normal, although no accurate counts and measurements were made. I at first considered this apparent atrophy as due to the poorer differentiation caused by the changes in the acinar cells, but they may represent the earlier and transitory atrophic changes noted by Sauerbeck, Ssobolew, and others, especially as in those animals that lived more than sixty days the areas appeared perfectly normal.

After some time, in some of my cases the duct became permeable again, as shown by passing a colored fluid through it into the duodenum, and in one case the enlarged and sclerosed duct broke through to the surface of the pancreas and so found vent for the secretion. In either case, there was a partial regeneration of the glandular tissue, and the regenerated tubules seemed to originate from the old, collapsed tubules that made up the atrophied lobule. The cells, though non-granular, flattened, non-functionating, and appearing like connective-tissue cells, had not lost the power of functionating and soon regained the appearance of secreting acini. In such glands, shown especially well in two experiments (No. XVI, 94 days and No. XXIII, 197 days), only two or three tubules with cells having granular protoplasm could be seen in a lobule section generally toward the center where the pressure of the connective tissue is less than in the outer part of the lobule, and where the rich blood supply of the areas of Langerhans may have helped to maintain the vitality of the cells. It was sometimes possible to trace a direct transition from the flattened and collapsed membrana propria which appeared merely as strands of connective tissue, through strands of cells containing a few granules to tubules staining like normal pancreatic acini. This easily and naturally explains the rapid regeneration of an extremely atrophied gland when an exit is made for its secretion. It may be to this method of regeneration that Ssobolew referred when he stated that in cats the ducts regenerated and the gland then soon regenerated, if the degeneration had not gone too far.

In most of the cases, I examined the urine during the first few days after the operation and again just before death. Sugar was not found in any of these cases, and as Sauerbeck's observations had not at that time appeared and I was unaware of the possible occurrence of glycosuria after the first month, I did not continue the examinations long enough to be able to speak upon this point. In four of my cases, I tried the Mayo-Robson test on the urine just before death, with positive results in three cases and negative in one.

In all cases except when spontaneous death occurred during the night, the pancreas was removed immediately after the death of the animal and small pieces taken, one from the ligatured end, one from the neighborhood of the ligature, and one from the free end, and fixed in corrosive-sublimate solution for microscopic study. The rest of the gland, usually in two portions, was extracted either with glycerine or with water and its digestive and glycolytic powers tested. Starch digestion was tested with iodin and with Fehling's solution. Fibrin digestion was determined by the use of the bromine tryptophan reaction and the biuret test. Fat digestion was indicated by the reaction and by emulsification. The glycolytic power was tested by following as nearly as possible Cohnheim's method: a definite amount of glucose was added to a definite amount of muscle extract, and to this were added the pancreatic extract and also large amounts of toluol for its bactericidal action. In some cases, both boiled and unboiled pancreatic extracts, were used with the muscle extract and with the same results. The quantity of sugar was estimated, at once after mixing and again after twenty-four hours in the warm oven, with the ammoniacal copper solution of Pavy. Control tests with muscle alone and with pancreatic extract alone were carried on at the same time.

The results of these various *tests* are briefly indicated in the following table in which O indicates the mental end (usually the ligatured end), while D indicates the duodenal end (the free and hencemore normal end), of the pancreas. It will be noted from this table that seven of the twenty cases in which physiologic tests were made (and this included six of the ten cases in which the gland tissue was nearly normal) showed no digestion of starch, fibrin, or fat, while in several others the digestive action was very much weakened. The diminution and absence of digestive action are proportionate to the atrophic condition of the glandular tissue. In none of the cases, however, was there any appreciable weakening of the glycolytic or activator power of this extract, even when the glandular tissue was apparently atrophied

| No. | Operation | Time | Glandular Parenchyma | Areas of Langerhans | Physiologic Tests | | | | Other Organs |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Starch | Fibrin | Fat | Sugar | |
| 1 | Ligature and section at *s*. | 18 hrs. | Normal | Normal | Not tested | | | | |
| 2 | Ligature, section, cauterization and omental insertion at *s*. | 3 days | Hemorrhage near ligature | Normal | O. Pos<br>D. Pos. | Pos.<br>Pos. | Pos.<br>Pos. | Pos.<br>Pos. | |
| 3 | Three ligatures. | 5 days | Vacuolation and compression of acini | Normal | O. Pos.<br>D. Pos. | Pos.<br>Pos. | Pos.<br>Pos. | Pos.<br>Pos. | |
| 4 | Same as No. 2 | 6 days | Dilatation of ducts; compression of acini | Normal | No tests made | | | | |
| 5 | Ligature and section. | 6 days | No examination | | No tests made | | | | |
| 6 | Two ligatures at *s*. | 7 days | Dilatation of duct; compression of acini | Small and poorly differentiated | No tests made | | | | |
| 7 | Ligature, division, and cauterization. | 8 days | Ducts dilated. Hemorrhage near ligature | Small; cells small and protoplasm degenerated | O. Pos<br>D Pos. | Pos.<br>Pos | Pos.<br>Pos. | Pos.<br>Pos. | |
| 8 | Ligatures at *s*. and *t*. | 9 days | Vacuolation and compression of acini; necrosis and inflammation | Areas normal but small | No tests made | | | | Liver; granular degen. |
| 9 | Ligature and section of duct at *s*. | 10 days | Congestion and dilatation of ducts; acini compressed | Normal | O. Neg.<br>D. Pos. | Neg.<br>Pos. | Neg.<br>Pos. | Pos.<br>Pos. | Normal |
| 10 | Ligature, section, cauterization and insertion of omentum at *s*. | 13 days | Hemorrhage near ligature | Normal | No tests made | | | | Fat necrosis and hemorrhage |
| 11 | Ligature at *t*. | 15 days | Atrophy and sclerosis of ligatured end | Some normal; some atrophied and sclerotic | No tests made | | | | Kidneys: cloudy swelling, congestion and hyalin casts; liver: congestion, granular degeneration |
| 12 | Ligature, section, cauterization, and insertion of omentum at *s*. | 16 days | Atrophy | Normal | No tests made | | | | |
| 13<br>14 | Ligature, section, and cauterization at *s*. | 27 days | Atrophy and sclerosis; dilatation of ducts | Normal | O. Pos.<br>D. Pos. | Pos.<br>Pos. | Pos.<br>Pos. | Pos.<br>Pos. | Normal |
| 15 | Ligature, section, cauterization, insertion of omentum at *s*. | 40 days | Extreme atrophy and sclerosis | Normal; seem increased | O. Neg.<br>D. Pos. | Neg.<br>Pos. | Neg.<br>Pos. | Pos.<br>Pos. | |
| 16 | Ligature at *t*. | 35 days | Atrophy and sclerosis; dilatation of duct | Normal | O. Pos.<br>D. Neg. | Pos.<br>Neg. | Pos.<br>Neg. | Pos.<br>Pos. | |
| 17 | Ligature, section, cauterization, insertion of omentum at *s*. | 59 days | Extreme atrophy and sclerosis; dilatation of duct | Normal except near ligature | O. Neg.<br>D. Pos. | Neg.<br>Pos. | Neg.<br>Pos. | Pos.<br>Pos. | |
| 18 | Ligature and section at *s*. | 87 days | Sclerosis but little atrophy | Normal | O. Pos.<br>D. Pos. | Pos.<br>Pos. | Pos.<br>Pos. | Pos.<br>Pos. | |
| 19 | Ligature at *t*. | 90 days | Nearly normal | Normal | Pos. | Pos. | Pos. | Pos. | |
| 20 | Ligature, section and cauterization at *s*. | 94 days | Extreme atrophy and selcrosis | Normal | O. Neg.<br>D. Pos. | Neg.<br>Pos. | Neg.<br>Pos. | Pos.<br>Pos. | |

| o. | Operation | Time | Glandular Parenchyma | Areas of Langerhans | Physiologic Tests. | | | | Other Organs. |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Starch | Fibrin | Fat | Sugar | |
| 21 | Ligature, section, and cauterization at *s*. | 100 days | Extreme atrophy and sclerosis | Normal | O. Neg.<br>D. Pos. | Neg.<br>Pos. | Neg.<br>Pos. | Pos.<br>Pos. | Liver : fatty degeneration |
| 22 | Ligature at *t*. | 112 days | Slight sclerosis and atrophy | Normal | Pos. | Pos. | Pos. | Pos. | Liver: granular and fatty |
| 23 | Ligature at *s*. | 114 days | Extreme atrophy and sclerosis | Normal | O. Neg.<br>D. Pos. | Neg.<br>Pos. | Neg.<br>Pos. | Pos.<br>Pos. | |
| 24 | Ligatures at *s*. and *t*. | 114 days | Regeneration; atrophy and sclerosis near ligature | Normal | No tests made | | | | |
| 25 | Ligature at *s* | 119 days | Atrophy and sclerosis | Normal | O. Pos.<br>D. Pos. | Pos.<br>Pos. | Pos.<br>Pos. | Pos.<br>Pos. | |
| 26 | Ligatures at *s* and *t*. | 126 days | Atrophy and sclerosis | Normal | O. Pos.<br>D. Pos. | Pos.<br>Pos. | Pos.<br>Pos. | Pos.<br>Pos. | |
| 27 | Ligature and section at *s*. | 156 days | Atrophy and sclerosis | Normal | O. Pos.<br>D. Pos. | Pos.<br>Pos. | Pos.<br>Pos. | Pos.<br>Pos. | |
| 28 | Ligature section, and cauterization at *s*. | 197 days | Extreme atrophy and sclerosis | Normal | O. Neg.<br>D. Pos. | Neg.<br>Pos. | Neg.<br>Pos. | Pos.<br>Pos. | |

The following table of the glycolytic tests in the seven cases in which there was no digestive action will indicate the very slight change, if any, in the activator power of the pancreatic juice caused by the extreme atrophy of the pancreatic acini. The slight difference is probably due to the smaller amount of the "activator principle" used in the case of the atrophied portion of the gland. (O, indicates omental and D, duodenal end of pancreas.)

| No. | Time. | Original amount of sugar in grm. | Amount of sugar after 24 hours in grm. | Actual loss in grm. | Per cent. of loss. |
|---|---|---|---|---|---|
| 15. | 49 days. | O 2.0776<br>D 2.0776 | 1.315<br>1.209 | 0.762<br>0.868 | .7%<br>.8% |
| 16. | 53 days. | O 4.25<br>D 4.25 | 3.67<br>3.57 | 0.58<br>0.68 | .6%<br>.7% |
| 17. | 59 days. | O 1.66<br>D 1.66 | 0.83<br>0.81 | 0.83<br>0.83 | .83%<br>.81% |
| 20. | 94 days. | O 0.76<br>D 1.13 | 0.4<br>0.77 | 0.36<br>0.25 | .12%<br>.1% |
| 21. | 100 days. | O 1.0125<br>D 0.875 | 0.27<br>0.215 | 0.74<br>0.66 | .8%<br>.9% |
| 23. | 114 days. | O 0.69<br>D 0.93 | 0.21<br>0.29 | 0.48<br>0.64 | .5%<br>.6% |
| 28. | 197 days. | O 1.5<br>D 1.13 | 1.15<br>0.77 | 0.25<br>0.36 | .41%<br>.42% |

The experiments have not proven so decisive as I had hoped, and for two reasons: (1) the isolation of the areas was not always complete; and(2) the insulæ in the cat's pancreas are generally small and it is difficult to get, even from the larger omental portion of the pancreas, when atrophied, sufficient extract to make very many satisfactory tests. I feel sure, however, that the chemical and physiological examination of isolated island tissue offers the only certain means of deciding the difficult questions regarding the function of these structures. While my results must be regarded as relative rather than absolute, they speak with no uncertain voice in favor of the theory that the islands manufacture a substance, analogous to the "activator principle" described by Cohnheim, which favors the glycolytic action of muscle ferment. To make these results more certain, it is necessary to use the isolated island tissue in large enough amounts so that numerous tests may be made not only of its activator action on muscle ferment, but also its effect on blood pressure and, most important of all, its influence on the experimental diabetes of depancreatized animals and on human diabetics. It is a question whether the operative method can in any animal furnish the isolated island tissue in large enough amounts for these purposes.

Rennie, however, has recently described two species of fish (*Lophius piscatorius* and *Scorpæna scropha*) in which one large "principal islet" and several smaller islands of structure analogous to that of the areas of Langerhans in mammalia may be distinguished with the unaided eye and may be dissected out entirely free and isolated from pancreatic tissue. If these shall prove to have the same function as the areas of Langerhans in mammalia and other forms, they offer the opportunity for further and more satisfactory investigation along these lines. Since my experimental work was finished and had been reported in part at the session of the Association of American Anatomists at Philadelphia in 1904, two preliminary reports have appeared, one by Rennie himself and one by Diamare and Kuliabko, announcing that they are now following out a similar line of investigation on the isolated islands of these two species of fish. The results given in their preliminary reports are by no means decisive or very satisfactory. Rennie reports three tests, two qualitative and one quantitative, on a glucose solution with an extract of these areas. The result would seem to show that this extract does not *alone* have the power of inverting grape sugar. He hints, however, that his experiments with this extract on diabetic patients have been more favorable to the island theory of diabetes. Diamare and Kuliabko, on the other hand, tested in several cases the action of this island extract on starch solution and on glucose solution. They were able to show, so far as their few tests made can decide, that these islands have no power whatever to digest starch. While the starch solution with the pancreatic extract from the same fish gave with iodine no starch reaction, and with Trommer's test gave marked sugar reaction, that with the island extract gave only the starch reaction with iodine. Their test for the glycolytic action is much less decisive, since no change was noted until after forty-eight hours, so that the inversion may have been due to bacterial action; further experiments, therefore, are necessary. The outline which they give of the work which they are now carrying on shows that they are carefully investigating the chemical and physiological characteristics of these isolated areas of Langerhans in the fish, and we shall hope for much light on this question when their work shall have been completed and their results published.

In conclusion it may be stated that there occur in the pancreas of all vertebrates homologous structures known as areas of Langerhans.

These have the structure of vascular glands with a sinusoidal circulation and are not changed during the secretory activity of the pancreas. They also remain unaltered when the glandular acini become atrophied as a result of ligation, section, or obstruction of the duct.

They have a secretion which is probably poured into the bloodvessels; this secretion has no digestive action on starch, fibrin, or fat, but has a marked glycolytic function, especially when added to muscle extract and is therefore analogous to the "activator principle" described by Cohnheim.

In closing I am glad to acknowledge my indebtedness to

Professor G. Carl Huber for many helpful suggestions received during the progress of this investigation.

## EXPLANATION OF PLATES.

### PLATE XII.

Fig. 1.—Wax reconstruction of typical island of Langerhans from pancreas of rat. × about 200.

Figs. 2 and 3.—Wax reconstructions of islands of Langerhans from pancreas of rabbit. P, connection with pancreatic tubules. × about 200.

### PLATE XIII.

Figs. 1 and 2.—Wax reconstructions of areas of Langerhans from pancreas of cat. × about 200.

Figs. 3, 4, 5, and 6.—Wax reconstructions of injected blood-vessels of areas of Langerhans from pancreas of cat. × about 200.

### PLATE XIV.

Fig. 1.—Wax reconstruction of area of Langerhans from human pancreas. × about 250.

Fig. 2.—Wax reconstruction of blood-vessels with surrounding connective tissue in same area, the cellular cords of which are reproduced in Fig. 1. × about 250.

### PLATE XV.

Fig. 1.—Interior view of model reproduced in Fig. 1, Plate XIV, Fig. 1. × about 200.

Fig. 2.—Interior view of model reproduced in Fig. 2, Plate XIV, Fig. 2. × about 200.

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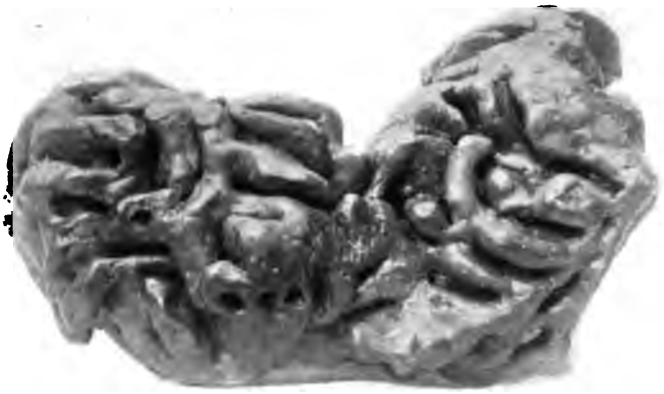

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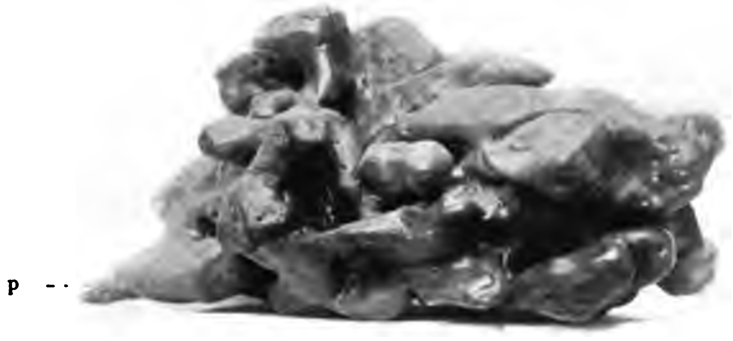

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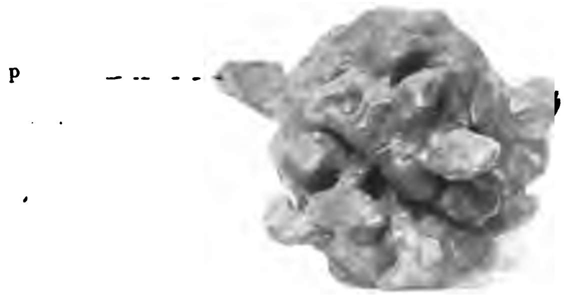

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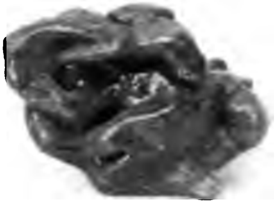

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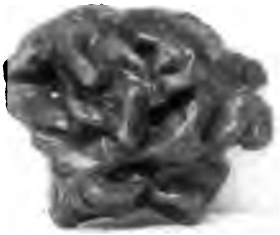

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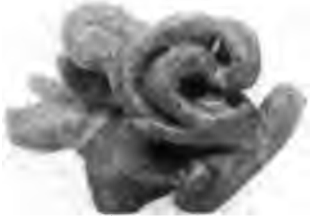

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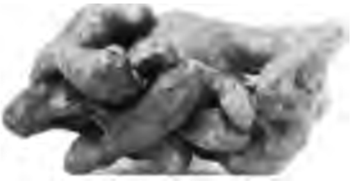

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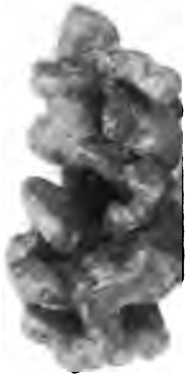

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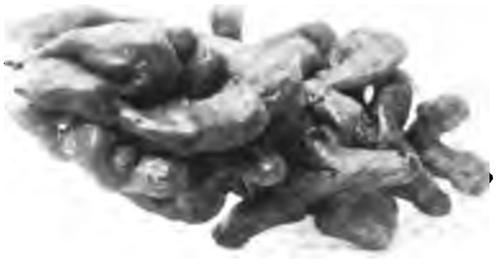

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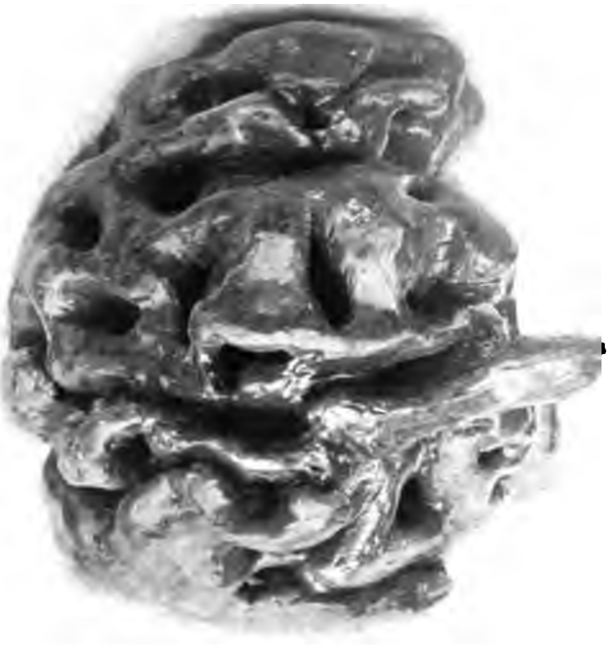

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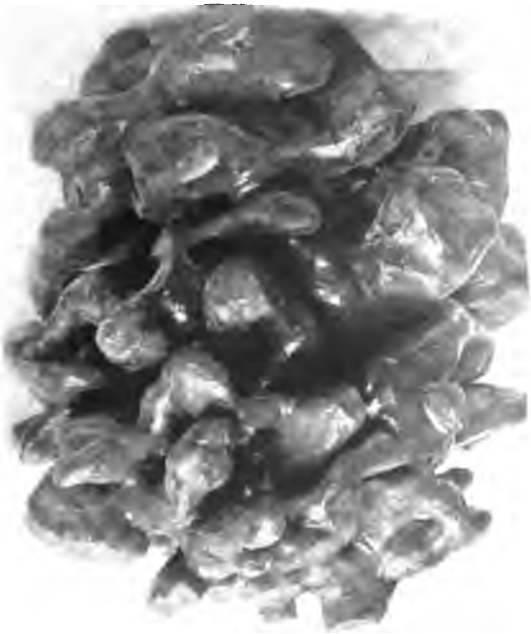

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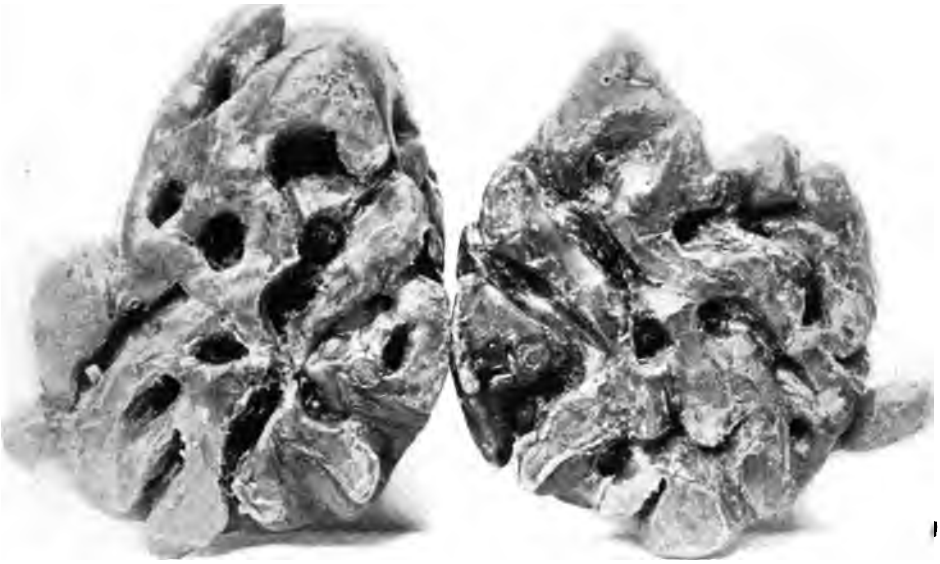

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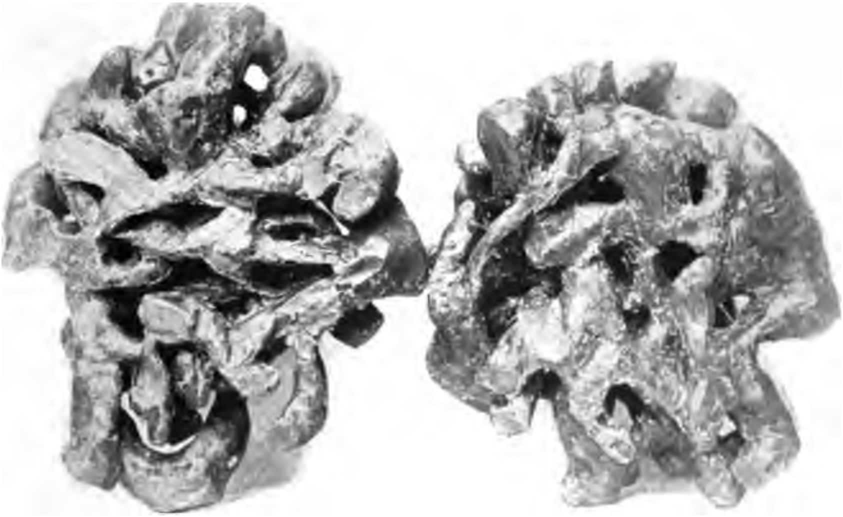

Fig. 2.

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# THE EFFECT OF INTRAVENOUS INJECTION OF SOLUTIONS OF DEXTROSE UPON THE VISCOSITY OF THE BLOOD.[1]

BY R. BURTON-OPITZ.

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The viscosity of the blood is expressed in this paper in terms of the coefficient K, obtained by calculation from the following known factors: the quantity of blood collected during a definite period, its specific gravity, the blood pressure, and the length and internal diameter of the capillary tube through which the blood is allowed to flow. When this coefficient has been compared with K for distilled water at 37° C., the value of which has been proved by Poiseuille to be 4700, it is possible to express the viscosity of the blood in terms of multiples of the viscosity of distilled water, heated to the temperature of 37° C. The method by means of which the aforesaid factors were obtained has been fully described by Hürthle.[2]

In the experiments now to be recorded, I have made use of four capillary tubes, numbered II, III, IV, and V. Tube No. II was used in Experiments 3, 4, 5, and 6; No. III in Experiment 1; No. IV in Experiment 2, and No. V in Experiment 7. The accuracy of each capillary was tested beforehand by a series of experiments with distilled water. Their dimensions were as follows:

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[1] This study has been conducted under a grant from the Rockefeller Institute for Medical Research.

[2] Hürthle, *K. Arch. für die ges. Phys.*, 1900, lxxxii, 415.

| Capillary | Length<br>mm. | Diameter<br>mm. |
|---|---|---|
| II | 213.0 | 0.6636 |
| III | 245.5 | 0.6636 |
| IV | 299.2 | 0.4100 |
| V | 331.7 | 0.7150 |

The experiments were performed upon dogs under light ether narcosis. The method remained the same in all cases. The normal viscosity having been tested by a number of determinations, a certain quantity of a concentrated solution of dextrose, heated to body temperature, was slowly injected into the facial vein. After an interval lasting from ten to fifteen minutes three or four additional determinations were made, fifteen minutes apart.

The details of the experiments with the concentrated solution of dextrose are given in Table I. In Experiments 1 and 2 only 5 c. c. of the solution were injected, while in the last two, 50 and 100 c. c. respectively were administered. The determinations in each experiment fall into two groups, which show respectively the normal viscosity and the viscosity prevailing after the injection.

On comparing the coefficients of the viscosity with one another, it is found first of all that their value does not remain constant. In the first two experiments, K became smaller after the injection, but in the third and fourth experiments larger than normal. As the actual change in the viscosity is inverse to the numerical change in the coefficient, these facts imply that the viscosity of the blood is increased by small and decreased by large quantities of the solution. The increase which occurred when the quantity of solution injected was small is only a moderate one, and is entirely in accord with the fact[3] that small amounts of pure distilled water, even when used alone, render the blood slightly more viscous. Larger quantities of the solution of dextrose, on the other hand, produced an im-

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[3] Burton-Opitz, R., *Jour. of Phys.*, 1904, xxxii, 8.

TABLE 1.

THE VISCOSITY OF THE BLOOD AFTER INTRAVENOUS INJECTION OF DEXTROSE.

| Number of exp. and weight of dog | | Deter. No. | Quantity of sol. inj. c. c. | Time elapsed since inj. min. | Temperature of animal °C. | Specific gravity of blood | Quantity mg. | Time sec. | Pressure mm. Hg. | Viscosity coefficient K | Difference between K of same group | Mean value of K | Change in K equals |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Exp. 1 13.0 K. | Normal | 1 | — | — | 36.8 | 1.0547 | 2615.1 | 18.24 | 179.8 | 957.24 | 59.46 | 986.97 | 103.70 |
| | | 2 | — | — | .. | | 1865.2 | 14.31 | 153.9 | 1016.70 | | | |
| | After inj. | 3 | 5 | 15 | .. | 1.0564 | 2092.7 | 16.71 | 174.2 | 861.60 | 51.70 | 883.27 | |
| | | 4 | — | 30 | .. | | 2510.4 | 20.53 | 167.5 | 874.93 | | | |
| | | 5 | — | 45 | .. | | 2374.4 | 18.30 | 170.2 | 913.30 | | | |
| Exp. 2. 17.5 K. | Normal | 1 | — | — | 36.5 | 1.0589 | 233.7 | 22.22 | 138.1 | 761.56 | 29.86 | 746.63 | 37.70 |
| | | 2 | — | — | .. | | 250.9 | 24.74 | 138.6 | 731.70 | | | |
| | After inj. | 3 | 5 | 15 | .. | 1.0594 | 168.8 | 22.53 | 106.8 | 701.14 | 15.59 | 708.93 | |
| | | 4 | — | 30 | .. | | 200.3 | 22.31 | 125.2 | 716.73 | | | |
| Exp. 3 12.0 K. | Normal | 1 | — | — | 37.0 | 1.0522 | 2662.9 | 17.46 | 154.8 | 1028.42 | 77.03 | 1035.69 | 51.24 |
| | | 2 | — | — | .. | | 2130.8 | 14.90 | 138.5 | 1077.85 | | | |
| | | 3 | — | — | | | 2568.0 | 18.60 | 144.0 | 1000.82 | | | |
| | After inj. | 4 | 50 | 15 | .. | 1.0504 | 2896.5 | 16.25 | 159.6 | 1167.80 | 146.35 | 1086.93 | |
| | | 5 | — | 30 | .. | | 3007.4 | 19.32 | 151.9 | 1071.54 | | | |
| | | 6 | — | 45 | .. | | 2686.3 | 20.25 | 135.8 | 1021.45 | | | |
| Exp. 4 13.0 K. | Normal | 1 | — | — | 36.8 | 1.0544 | 1915.0 | 20.07 | 115.4 | 861.26 | 41.83 | 851.14 | 95.92 |
| | | 2 | — | — | .. | | 2016.3 | 19.84 | 122.1 | 867.00 | | | |
| | | 3 | — | — | .. | | 1685.9 | 18.41 | 115.6 | 825.17 | | | |
| | After inj. | 4 | 100 | 10 | .. | 1.0519 | 2471.7 | 21.54 | 121.1 | 989.38 | 79.28 | 947.06 | |
| | | 5 | — | 20 | .. | | 2724.7 | 25.43 | 118.8 | 941.70 | | | |
| | | 6 | — | 30 | .. | | 2362.7 | 23.53 | 115.2 | 910.10 | | | |

mediate decrease corresponding to the amount of solution injected. The specific gravity of the blood pursued, in these experiments, a course parallel to the vicosity.

It is also to be noted that the viscosity did not retain its new value for any length of time, but assumed with each successive determination a value closer to the normal. The greatest change was observed, therefore, shortly after the injection, while thirty to forty-five minutes later, when osmotic interchanges between the blood and the tissues had taken place, the difference had become much less evident.

Three additional experiments were performed with a view to determine the viscosity during hyperglycæmia, this condition having been brought about by painting the surface of the pancreas with a solution of adrenalin. A number of determinations of the viscosity were made before as well as after the adrenalin had been applied. In all three cases traces of sugar were found in the urine after the completion of the last determination. The details of these experiments can be readily obtained from Table II.

It is clearly brought out in the foregoing table that the viscosity of the blood during hyperglycæmia is somewhat greater than normal; however, the increase did not amount to more than seventy points in the experiments now recorded. Thus, if Experiment 7 be taken as an example, we find that the viscosity, which was in this case 6.6 times as great as that of distilled water at 37° C., became 7.0 times as great after the use of the adrenalin. Corresponding changes were noted in the specific gravity of the blood.

TABLE II.

THE VISCOSITY OF THE BLOOD AFTER APPLICATION OF ADRENALIN TO PANCREAS.

| Number of exp. and weight of dog | | No. of determ. | Temp. of animal | Specific gravity of blood | Quantity mg. | Time sec. | Pressure mm. Hg. | Viscosity coefficient K | Difference between K of same group | Mean value of K | Change in K equals: |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Exp. 5 | Normal | 1 | 36.5 | 1.0488 | 1333.4 | 13.94 | 96.1 | 1042.35 | 39.85 | 1062.95 | 53.48 |
| | | 2 | " | | 1853.2 | 15.54 | 115.4 | 1082.20 | | | |
| | | 3 | " | | 1672.3 | 14.51 | 113.4 | 1064.32 | | | |
| 9.5 K. | After applic. of adrenalin | 4 | " | 1.0494 | 1778.7 | 16.15 | 113.5 | 1015.60 | 16.50 | 1009.47 | |
| | | 5 | " | | 2338.8 | 17.94 | 134.6 | 1013.72 | | | |
| | | 6 | " | | 128.59 | 15.83 | 85.1 | 999.10 | | | |
| Exp. 6 | Normal | 1 | 36.5 | 1.0610 | 1900.9 | 14.37 | 171.1 | 800.32 | 22.05 | 801.83 | 47.37 |
| | | 2 | " | | 2269.6 | 14.96 | 189.5 | 813.62 | | | |
| | | 3 | " | | 1632.0 | 15.18 | 140.6 | 791.57 | | | |
| 10.0 K. | After applic. of adrenalin | 4 | " | 1.0614 | 1462.7 | 12.08 | 165.4 | 757.55 | 6.17 | 754.46 | |
| | | 5 | " | | | | | | | | |
| | | 6 | " | | 1482.0 | 12.20 | 167.3 | 751.38 | | | |
| Exp. 7 | Normal | 1 | 36.9 | 1.0559 | 1100.5 | 16.54 | 111.1 | 719.85 | 14.87 | 710.02 | 66.32 |
| | | 2 | " | | 1344.5 | 18.31 | 125.2 | 704.98 | | | |
| | | 3 | " | | 1631.6 | 13.40 | 207.5 | 705.23 | | | |
| 9.5 K. | After applic. of adrenalin | 4 | " | 1.0573 | 1511.2 | 15.84 | 183.8 | 623.11 | 34.43 | 643.70 | |
| | | 5 | " | | 1582.1 | 15.99 | 182.6 | 650.46 | | | |
| | | 6 | " | | 1399.1 | 16.64 | 153.5 | 657.54 | | | |

# EFFECTS OF INTRAVENOUS INJECTIONS OF RADIUM BROMIDE.

BY R. BURTON-OPITZ AND GUSTAVE M. MEYER.

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PLATE XVI.

The present study was undertaken with a view of determining in a general way the effects of intravenous administration of radium upon the circulation and respiration. The problem was suggested to us by Dr. William J. Gies, under whose guidance a number of researches, dealing with the more extensive question of the action of radium upon animal and vegetable cells, have recently been carried on in the laboratories of Columbia University.[1]

For the radium used in these experiments we are greatly indebted to Mr. Hugo Lieber. It was supplied to us in the form of the bromide, in preparations of 240, 1000, and 10,000 activities. The strength of the solution used was the same in all cases. It contained 45 mg. of the dry substance in 25 c. c. of the solvent; each cubic centimeter of the solution, therefore, contained 1.8 mg. of the radium preparation.

The amount of the radium present varies directly with the radio-activity. Preparations of 1,500,000 activity are said to represent pure radium bromide.[2] Taking this figure as the standard of purity, 1.8 mg. of the radium preparation of 10,000 activity contained approximately only 0.0126 mg., the same quantity of the preparation of 1000 activity contained 0.00126 mg. and a like amount of the preparation of 240 activity only 0.00036 mg.

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[1] Preliminary communications of the results were made last May. See Gies and collaborators: *Proceeding of the Society for Experimental Biology and Medicine*, 1905, ii, 86; also, *Science*, 1905, xxi, 986; *American Medicine*, 1905, ix. 1026; *Medical News*, 1905, lxxxvii, 520.

[2] Rutherford, *Radio-activity* (Second Edition), 1905, p. 14, "No definite results have yet been given on the activity of pure radium, but the Curies estimate that it is about one million times that of uranium and may possiby be higher."

of actual radium bromide. Expressed in terms of the element radium (atomic weight = 225), each cubic centimeter of the solutions of the different preparations used in these experiments represents respectively about 0.00739, 0.00074, and 0.000178 mg. of radium. It must also be mentioned beforehand that the effect of these radium preparations may be due not alone to the radium, but to its disintegration products, namely, emanation,—radium A, radium B, radium C — or to the radiations given off by these. As will be brought out later, there appears to be a distinct difference between the action of the perfectly fresh solution and one that has been left standing for some time in an open receptacle. Whether this difference is due to an increased or decreased amount of emanation in the solution has not been determined as yet.

The substance was dissolved in distilled water, perfectly fresh solutions, heated to body temperature, being employed for the injections. The experiments were performed upon dogs weighing from three to seven kilos. Light ether narcosis was employed. The blood pressure was recorded by a mercury manometer connected with the carotid artery and the respiratory movements by means of a modified pneumographic arrangement. The injections were made into the facial vein.

A number of preliminary tests were made to determine the influence of the intravenous injections of such quantities of distilled water as were made use of subsequently in the experiments with radium bromide. Only in those cases in which the injection was made with more than a moderate degree of rapidity could any effect upon the circulation be detected.

The radium bromide of 240 activity was employed in six experiments, approximately 10 c. c. of the solution, or 18 mg. of the preparation being administered in each case. Curve I, (Plate XVI) which forms only a small part of the entire record of Experiment I, may serve to show the character of the changes observed. It was obtained from a dog weighing four kilos and differs in no particular from the other records of this series. The injection began at A and was discontinued at B. Besides the blood pressure P; the curve also includes a record of the respiratory movements R and a time-tracing Sec.

The blood pressure began to rise in all cases shortly after the beginning of the injection and continued to increase gradually until about 5 c. c. of the solution had been injected, or during a period of from thirty to thirty-five seconds. The length of the period intervening between the beginning of the injection and the appearance of the rise depended somewhat upon the rapidity with which the solution was injected. On the whole, however, it was rather brief, averaging about four or five seconds. The rise itself appeared as a rule rather abruptly and attained its maximum value in the course of about thirty-five seconds. The maximum increase here recorded amounted to 60 mm. Hg and the minimum rise to 20 mm. Hg, the average noted in the six experiments being 35 mm. Hg.

By comparing the normal frequency of the heart with its rate during the period of increased blood pressure, only very insignificant differences were brought out. If anything, a hardly recognizable inhibition had taken place, which, however, was more than compensated for by an increase in the force of the cardiac contractions. A study of the curves proves beyond question that radium bromide of this activity exerts a moderate tonic action upon the heart muscle. However, the greater force of the contractions is not at all sufficient to explain the marked increase in blood pressure. For this change, no doubt, a direct vaso-constrictor influence of the radium bromide upon the musculature of the blood-vessels must be held responsible.

The gradual rise in blood pressure, caused, as has just been stated, by a general constriction of the blood-vessels, was invariably interrupted after about thirty-five seconds by a very pronounced irregularity and inhibition of the heart. Relatively long diastolic periods were now followed in rapid succession by periods during which a number of short forcible contractions caused the mercury to rise far beyond any ordinary level, but in spite of the fact that the blood pressure attained at times quite extraordinary heights, its average value remained as a rule well below the height to which it had risen during the period of vaso-constriction. As the experiment progressed, the pressure gradually became lower and lower and sank to zero shortly after the onset of the respiratory paralysis. It happened at times that the heart regained for a period of indefinite length a certain regularity of contraction, but even during these moments of partial restoration its frequency was very much less than normal.

A change in the rate of respiration during the period of vaso-constriction could not be detected. Shortly after the onset of the cardiac symptoms, however, the respiratory movements became distinctly less frequent and deep. As the experiment progressed, this inhibitory influence came more and more into prominence, until finally a complete respiratory paralysis resulted. The heart ceased beating shortly after the respiratory movements came to a standstill. When the lungs were inflated artificially soon after the onset of the respiratory paralysis, the heart action continued; respiration, on the other hand, did not become automatic again during the time of these experiments, i. e., within about ten minutes after the cessation of the natural respiratory movements.

Two experiments were made in addition upon dogs whose vagi had been divided beforehand. A difference in the effect of the radium bromide, directly ascribable to this operative measure, could not be detected, excepting, of course, a lesser prominence of the initial vaso-constrictor reaction. The latter change was due entirely to the pre-existing high arterial tension and blood pressure. Vagus stimulation remained effective throughout the experiment. These facts, therefore, tend to prove that the radium bromide does not exert a central, but rather a direct influence upon the substance of the heart.

Several tests were also made with radium bromide solutions that had been left standing in an open flask for about one week preceding the day of the experiment. It was found in all cases that these solutions had lost a good deal of their effectiveness.

The details of the experiments with radium bromide of 240 activity are given in the following table in conjunction with those in which the preparations of 1000 and 10,000 activities were employed. Besides the weight of the animal and the amount of radium administered in each case, the table also gives comparative figures regarding the cardiac and respiratory frequency and the blood pressure during the different periods of the experiment.

THE EFFECT OF INTRAVENOUS INJECTION OF RADIUM BROMIDE SOLUTIONS.

| | Exp. | Weight of dog K. | $RaBr_2$ injected. per mg.K | Normal | | | Period of constriction | | | Period of irregular heart action | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Resp. rate | Pulse | Blood pr. | Resp. rate | Pulse | Blood pr. | Reps. rate | Pulse | Blood pr. |
| **$RaBr_2$** **240 act.** | 1 | 3.4 | 5.3 | 9 | 90 | 88.6 | 8½ | 90 | 108.9 | cessation | very irreg; | cess. |
| | 2 | 5.0 | 3.6 | 12 | 85 | 77.5 | 12 | 85 | 121.0 | “ | “ “ | “ |
| | 3 | 3.7 | 4.9 | 9 | 74 | 122.5 | 10 | 75 | 182.8 | “ | “ “ | “ |
| | 4 | 4.3 | 4.2 | 11 | 78 | 103.7 | 11 | 76 | 146.0 | “ | “ “ | “ |
| | 5 | 5.7 | 3.2 | 8 | 95 | 126.2 | 8 | 89 | 152.1 | “ | “ “ | “ |
| | 6 | 4.6 | 3.9 | 12 | 82 | 114.9 | 12 | 82 | 139.3 | very slow | “ “ | “ |
| **$BaBr_2$** **1000 act.** | 7 | 4.0 | 2.5 | 14 | 80 | 136.8 | 13 | 78 | 169.0 | cessation | “ “ | “ |
| | 8 | 5.5 | 3.9 | 12 | 76 | 132.7 | 12 | 76 | 152.2 | “ | “ “ | “ |
| **$RaBr_2$** **10,000act.** | 9 | 5.5 | 3.3 | 16 | 82 | 140.1 | 17 | 82 | 175.7 | 9 | 36 | 87.6 |
| | 10 | 5.2 | 3.6 | 17 | 86 | 114.8 | 17 | 82 | 126.5 | 10 | 47 | 102.2 |
| | 11 | 7.0 | 3.6 | 13 | 91 | 97.7 | 14 | 91 | 132.1 | 8 | 31 | 88.7 |
| | 12 | 5.5 | 3.4 | 18 | 80 | 122.1 | 17 | 80 | 135.6 | 8 | 41 | 75.2 |

As barium bromide forms so large a part of the radium bromide preparations, it was thought possible that the effects noticed in the preceding experiments might be due at least in part to this constituent. To test this assumption several experiments with pure barium bromide solutions were performed, the method being the same as the one described in an earlier paragraph of this paper.

Our results relative to the barium are entirely in harmony with those described by Mickwitz,[3] Böhm,[4] and Bary.[5] Differences in the degree of the reaction were observed by the first two authors in accordance with the strength of the barium solution employed. When small doses were given (0.01 gm.) only slight rises in the blood pressure were observed. After larger doses (0.02 gm.) the increase in the blood pressure was followed by an irregularity of the heart, while still larger amounts (0.04 gm.) caused in addition a rapid fall in blood pressure and the death of the animal. Bary corroborated these results and showed that the rise in blood pressure is due to a direct action of the barium upon the musculature of the blood-vessels and a slight tonic action upon the cardiac muscle.

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[3] Mickwitz, Dissertation, Dorpat, 1874.

[4] Böhm, *Archiv f. exper. Path., u. Phar.*, 1875, iii, 216.

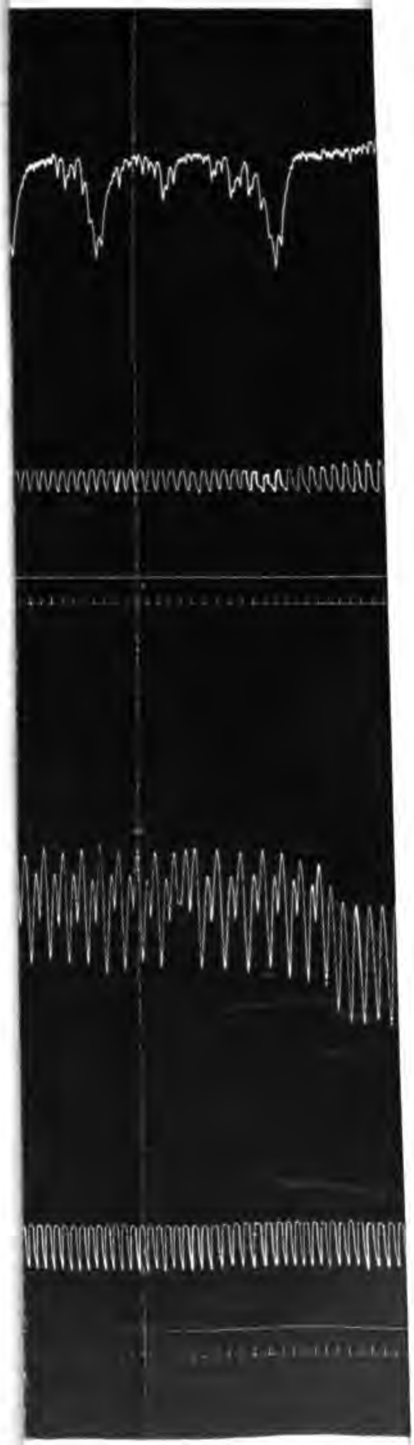
[5] Bary, Dissertation, Dorpat, 1888.

However, in order to bring the great similarity in the action of barium bromide and radium bromide of this activity more convincingly to the attention of the reader without entering upon a lengthy description, it has been thought best to reproduce underneath Curve I a part of a tracing obtained during one of the experiments with barium. This record does not differ in any important particular from those of the other barium experiments and, moreover, on comparing it with Curve I, it would be difficult to detect fundamental points of difference in the action of these two elements.

It is entirely probable, therefore, that the effects previously attributed to the radium preparation are due in a large measure to its main constituent, barium. In accordance with this assumption we might, moreover, suppose that these circulatory and respiratory changes would be less evident after the administration of radium of higher activity, unless radium showed more markedly the effects exhibited by barium, a possibility suggested by the chemical similarity of these two elements.

To test this inference, we performed, in addition, two experiments with radium bromide of 1000 activity and four experiments with radium bromide of 10,000 activity; proportionately equal amounts of the preparation were injected.

The results derived from the experiments with the radium of 1000 activity differed practically in no way from those obtained with the radium of 240 activity. Very definite changes were observed, however, when the preparation of 10,000 activity was employed. The initial vaso-constrictor reaction lost a large share of its former conspicuousness and abruptness, while the series of short forcible contractions of the heart which often caused the blood pressure to rise suddenly in jerks beyond any ordinary level were now replaced by slow pulsations of nearly equal amplitude. The average blood pressure remained well below the normal. The most prominent symptoms, therefore, were the vaso-constriction followed shortly afterward by a marked



cardiac inhibition, but without any marked irregularity of contraction. The inhibition began during the injection of the solution and continued for an indefinite period, a complete restoration of heart action being the usual result. These effects could be produced a second and third time by renewed injections of the solution; it was found, however, that constantly larger quantities had to be used, a fact favoring the view that a certain adaptation of the body to the radium bromide does take place.

The injections were also followed by a moderate decrease in the frequency and depth of respiration, the respiratory inhibition being most pronounced during the period of cardiac inhibition. However, a complete respiratory paralysis, as in the previous experiments, did not result.

While the injections of radium bromide of 240 and 1000 activities proved fatal with only one exception, the radium bromide of 10,000 activity did not produce fatal results within the time of the experiments. It seems to be established, therefore, that the injurious effects of the radium bromide preparations used were due in a large measure to the accompanying barium salt,—at least, the evidence here brought out points to that conclusion. Naturally, complete proof of this statement can be obtained only by the use of chemically pure radium salts, i. e., such as show activities of 1,000,000 or more.

# THE PHOTODYNAMIC ACTION OF EOSIN AND ERYTHROSIN UPON SNAKE VENOM.

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The study of the destructive action of photodynamic substances upon cells, toxins, ferments, and other chemical bodies, has opened up an interesting field of biological and chemical research. This paper will deal with the effects of two active photodynamic chemicals—eosin and erythrosin—upon the toxic principles of venom.

The starting-point of this study is the complex nature of snake venom, with its several independent toxic principles The effects of these principles are clearly demonstrable by biological tests *in vitro* and in the animal body. Since the action of snake venom has been extensively and profitably studied, the properties of the principles have been fairly established.[1] As regards their chief activities these principles can be divided into the following: neurotoxines, hæmolysins, hæmagglutinins, cytotoxines, hæmorrhagin, thrombokinase, and precipitin.[2] They differ not only in physiological action and chemical composition, but they show widely different labilities.

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[1] Mitchell and Reichert, *Smithsonian Contributions*, 1886, No. 647; Cunningham, *Scientific Memoirs by the Medical Officers of the Army of India*, 1895, Part IX, and 1898, Part XI; Kanthack, *Report of Medical Officers of Local Government Board*, London, 1895–1896; Stephens and Myers, *Journ. of Path. and Bact.*, 1899–1900, xi, 415; Stephens, *idem*, p. 273; Flexner and Noguchi, *Journ. of Exp. Med.*, 1902, vi, 277; *Journ. of Path. and Bact.*, 1905, x, 111; *Univ. of Penna. Med. Bulletin*, 1902, xv, 360; Noguchi, *Journ. of Exp. Med.* 1905, vii, 191; Mitchell and Stewart, *Mem. of National Acad. of Sciences*, 1898, viii; Kyes, *Berl. klin. Woch.*, 1903, xl, 956, 982; Kyes and Sachs, *idem*, 1903, xl, 21, 57, 82; Lamb, *Indian Med. Gazette*, 1901, xxxvi, 443; Martin, C. J., *Proc. Roy. Soc. of N. S. Wales*, 1892, xxvi, 240.

[2] This is the globulin and hæmoglobin precipitating body described by me. *Journ. of Exp. Med.*, 1905, vii, 191.

If the variation in resistance of these principles to moist heat is taken the order will be about as follows: neurotoxin resists brief boiling; hæmolysin is destroyed at 135° C., hæmagglutinin at 75° to 80°C.; hæmorrhagins, cytolysins, and thrombokinase at 75° C.; precipitin at 96° to 100° C. Now since the venoms of different species and orders of snakes vary according to the prevalence of one or the other class of toxic constituents, the ease with which they succumb to heating depends on the nature of the predominant principles. Hence rattlesnake venom in which hæmorrhagin and possibly other locally acting non-heat resisting poisons are predominant, and daboia venom in which much thrombokinase is contained, are easily diminished in activity by heating to 75° C., at which temperature cobra venom suffers little change in toxicity.

The venoms of the cobra, *Crotalus adamanteus*, and *Daboia Russellii* were exposed to the action of eosin and erythrosin. These anilines were chosen because of their pronounced action upon ferments and toxins.

*Action upon the Hæmolytic Principle.*—The venoms were employed in the following strengths: daboia, 0.1 per cent.; cobra and rattlesnake, 0.4 per cent. The dyes were used in 0.25 per cent. solution. Four parts of the venom solution were mixed with one part of the aniline solution. Hence they contained 0.05 per cent. of the dye. The mixtures were divided into two parts, one of which was kept in the dark while the other was exposed to sunlight for thirty hours. The controls consisted of venom solutions of the same strength in salt solutions kept under identical conditions as the dye mixture. Dog's blood in 3 per cent. suspension was employed. The total volume in each tube was two cubic centimeters; the reading was made after two hours at 37° C. and overnight at room temperature.

TABLE I.

COBRA HÆMOLYSIN.

| Venom solution in c.c. | Control | | Eosin | | Erythrosin | |
|---|---|---|---|---|---|---|
| | Dark | Exposed | Dark | Exposed | Dark | Exposed |
| 0.1 | C.H. | C.H. | C.H. | C.H. | C.H. | C.H. |
| 0.05 | " | " | " | Almost C.H. | " | " |
| 0.02 | " | " | " | " | " | " |
| 0.01 | " | " | " | Moderate H. | " | Almost C.H. |
| 0.005 | " | Almost C.H. | Almost C.H. | Slight H. | " | " |
| 0.002 | Moderate H. | Moderate H. | Moderate H. | Trace H. | Moderate H. | Moderate H. |
| 0.001 | " | " | " | No. H. | " | " |
| 0.0005 | " | " | " | " | " | " |
| 0.0002 | Slight H. | Slight H. | Slight H. | " | Slight H. | Slight H. |
| 0.0001 | Trace H. | Trace H. | Trace H. | " | Trace H. | Trace |
| 0.00005 | " | " | " | " | " | " |
| 0.00002 | " | " | " | " | " | " |
| 0.00001 | " | " | " | " | " | " |
| 0.000005 | " | " | " | " | " | " |
| 0.000002 | No. H. | No. H. | No. H. | " | No. H. | No. H. |
| 0.000001 | " | " | " | " | " | " |
| Control | " | " | " | " | " | " |

A glance at Table I shows that the hæmolysin of cobra venom is reduced in activity very little by exposure to sunlight in the presence of eosin and not at all in the absence of the dye or in the presence of erythrosin. The hæmolysin is, therefore, resistant to the photodynamic action of these two fluorescent bodies. In view of this result it was to be expected that cobra-lecithid prepared by Kyes's method should remain unaffected, a fact which points not only to the relative resistance of the hæmolysin but also to the stability of the compound. A similar series of tests with Crotalus venom gave different results.

The hæmolytic power of Crotalus venom is reduced by almost 200 times the original strength when measured by absence of hæmolysis, and by almost forty times when measured by slight hæmolysis. The dyes are without influence in the dark and exposure to direct sunlight for the period of the experiment does not injure the hæmolysin in the colorless solution.

TABLE II.

CROTALUS HÆMOLYSIN.

| Venom solution in c.c. | Control | | Eosin | | Erythrosin | |
|---|---|---|---|---|---|---|
| | Dark | Exposed | Dark | Exposed | Dark | Exposed |
| 0.2 | C.H. | C.H. | C.H. | Almost C.H. | C.H. | Almost C.H. |
| 0.1 | " | " | " | Moderate H. | " | Moderate H. |
| 0.05 | Almost C.H. | Almost C.H. | Almost C.H. | " | Almost C.H. | " |
| 0.02 | Much H. | Much H. | Much H. | Slight H. | Much H. | Slight H. |
| 0.01 | Moderate H. | Moderate H. | Moderate H. | Trace H. | Moderate H. | Trace H. |
| 0.005 | " | " | " | No. H. | " | " |
| 0.002 | " | " | " | " | " | No. H. |
| 0.001 | " | " | " | " | " | " |
| 0.0005 | Slight H. | Slight H. | Slight H. | " | Slight H. | " |
| 0.0002 | " | " | " | " | " | " |
| 0.0001 | " | " | " | " | " | " |
| 0.00005 | Trace H. | Trace H. | Trace H. | " | Trace H. | " |
| 0.00002 | " | " | " | " | " | " |
| 0.00001 | No H. | No. H. | No. H. | " | No. H. | " |
| 0.000005 | " | " | " | " | " | " |
| Control | " | " | " | " | " | " |

Cobra and Crotalus hæmolysins exhibit, therefore, a wide difference as regards their susceptibility to injury by eosin and erythrosin. This difference is in keeping with the labilities which they show in general. It is, therefore, of interest to ascertain whether the hæmolysin of daboia venom is to be placed with the former or the latter in its reaction to these substances. Table III which gives the results of the test with daboia venom shows it to occupy an intermediate position since its loss of power fluctuates between $\frac{1}{6}$ and $\frac{1}{10}$ according to the level of hæmolysis at which it is measured. With both rattlesnake and daboia venom eosin appears to be a little more active than erythrosin, but the differences are slight.

*Effects on the General Toxicity.*—The three venoms—cobra, Crotalus, and daboia—used in testing the effect of eosin and erythrosin upon the general venom toxicities sufficed to bring out the relative degrees of resistance of the neurotoxic, hæmorrhagic, and blood-coagulating principles. The toxic action of cobra venom being due mainly to the first principle, any marked reduction in toxicity which might take place would be ascribable to changes in that substance, and the same reasoning is applicable to hæmorrhagin as represented in rattlesnake venom and coagulin in daboia venom.

## TABLE III.

### DABOIA HÆMOLYSIN.

| Venom solution in c. c | Control | | Eosin | | Erythrosin. | |
|---|---|---|---|---|---|---|
| | Dark | Exposed | Dark | Exposed | Dark | Exposed |
| 0.3 | C.H | C.H. | C.H. | C.H. | C.H. | C.H. |
| 0.2 | " | " | " | " | " | " |
| 0.1 | " | " | " | " | " | " |
| 0.05 | " | " | " | " | " | " |
| 0.02 | " | " | " | " | " | " |
| 0.01 | " | " | " | Almost C.H. | " | " |
| 0.005 | " | " | " | " | " | Almost C.H. |
| 0.002 | Almost C.H. | Almost C.H. | Almost C.H. | Moderate H. | Almost C.H. | Much H. |
| 0.001 | " | " | " | " | " | Moderate H. |
| 0.0005 | " | " | " | Slight H. | " | Slight H. |
| 0.0002 | Much H. | Much H. | Moderate H. | " | Much H. | " |
| 0.0001 | " | " | " | Trace H. | " | Trace H. |
| 0.00005 | Slight H. | Slight H. | Slight H. | No. H. | Slight H. | " |
| 0.00002 | " | " | " | " | " | No. H. |
| 0.00001 | " | " | Trace H. | " | " | " |
| 0.000005 | No. H. | No. H. | No. H. | " | No. H. | " |
| 0.000002 | " | " | " | " | " | " |
| 0.000001 | " | " | " | " | " | " |
| Control | " | " | " | " | " | " |

The technique of the tests is simple. Stock solutions of venom are mixed with the dyes in solutions. The mixtures are divided into two parts one of which is exposed and the other kept in the dark. A simple solution of the same strength without any admixture is treated in the same manner—one half being exposed and the other placed in a dark chamber. The exposure to sunlight was, as a rule, for thirty hours.

*Cobra Venom*—0.2 per cent., stock solution 4 parts; 0.25 per cent., eosin or erythosin 1 part. All solutions made in 0.9 per cent. salt. Exposure 30 hours. Toxicity tested in guinea-pigs weighing 350 grams. Intraperitoneal injections.

## TABLE IV.

### COBRA VENOM AND GUINEA-PIGS.

| Venom in grm. | Control | | Eosin | | Erythrosin | |
|---|---|---|---|---|---|---|
| | Dark | Exposed | Dark | Exposed | Dark | Exposed |
| 0.001 | + 1 h. 25 m. | | | +1 h. 30 m. | | + 45 m. |
| 0.00075 | + 4 h. 50 m. | | | | | |
| 0.0005 | + 2 h. 48 m. | | | + 3 h. | | + 4 h. 10 m. |
| 0.0003 | + 3 h. 34 m. | | | | | |
| 0.00025 | + 6 h. | | | | | |
| 0.0002 | + 12 h. | + 18 h. | + 12 h. | + 24 h. | + 18 h. | +24 h. |
| 0.00015 | + 18 h. | + 36 h. | + 2 days | + 2 days | + 38 h. | +40 h. |
| 0.0001 | § | § | § | § | § | § |
| 0.000075 | § | § | § | § | § | § |
| 0.00005 | § | § | | | | |

+ =death. § = Survived.

The conclusion to be drawn from this series of tests is that the two anilines employed are without marked effects in diminishing the toxicity of cobra venom for guinea-pigs. On the other hand, with rabbits, a slight reduction in toxicity after the eosin treatment is apparent.

### TABLE V.

#### COBRA VENOM AND RABBITS.

Rabbit No. 1. Venom 0.002 gram. Death in 20 minutes.
Rabbit No. 2. Venom 0.002 gram + eosin 0.05% (Dark). Death in 20 minutes.
Rabbit No. 3. Venom 0.002 gram + eosin 0.05% (Sunlight). Slightly ill; recovered.
Rabbit No. 4. Venom 0.003 gram + eosin as before (Sunlight). Death in 80 minutes.
Rabbit No. 5. Venom 0.004 gram + eosin as before (Sunlight). Death in 15 minutes.
Rabbit No. 6. Venom 0.008 gram + eosin as before (Sunlight). Death in 60 minutes.

The reduction in toxicity as measured on rabbits probably does not exceed ⅓ and may be less, since the individual variation in susceptibility of the animals may affect the results. That such a factor operates at times is to be seen by comparing the results with Rabbits Nos. 5 and 6.

*Crotalus Venom.*—The preponderance of hæmorrhagin and the small quantity of neurotoxin contained in rattlesnake venom make it peculiarily suitable for tests of relative stability of these two classes of bodies. The symptoms and lesions of poisoning by rattlesnake venom can readily be interpreted. Hence any marked reduction in toxicity can be accounted for by the destruction of one or both of the chief toxic principles.

The venom was in 0.4 per cent. and the eosin and erythrosin in 0.25 per cent. solutions. The details of the method were the same as with cobra venom. Guinea-pigs of 250 grams were used for the tests, the injections being made into the peritoneum.

TABLE VI.

CROTALUS VENOM AND GUINEA-PIGS.

| Venom in grm. | Control | | Eosin | | Erythrosin | |
|---|---|---|---|---|---|---|
| | Dark | Exposed | Dark | Exposed | Dark | Expos |
| 0.03 | | | | + 5 days* | | + 4 day |
| 0.024 | | | | + 15 days* | | § |
| 0.018 | | | | No symptoms† | | No sympto |
| 0.012 | | | | “ | | .. |
| 0.008 | | | | “ | | .. |
| 0.004 | | | | “ | | .. |
| 0.001 | + 6 h.# | + 5 h.40 m.# | + 8 h.# | “ | + 10 h.# | .. |
| 0.0008 | + 8 h. | + 12 h. | | | + 15 h. | |
| 0.0006 | + 10 h. | + 24 h. | + 12 h. | | + 10 h. 50 m. | |
| 0.0005 | + 24 h. | + 3 days | + 24 h. | | + 24 h. | |
| 0.0004 | § | § | § | | § | |
| 0.0003 | § | § | § | | § | |

+ = Death.
# = Very marked hæmorrhage in the peritoneal cavity, viscera, and muscles.
* = Died of emaciation, no hæmorrhage.
† = Killed with chloroform and the peritoneal cavity examined after 48 hours; hæmorrhage.
§ = Survived.

From this table it is seen that it is the hæmorrhagic principle that is destroyed by the dyes after exposure to sunlight. However, the neurotoxin would also appear not to be left wholly intact; but to what change in action of the venom the marasmic condition which developed in certain of the pigs is due is not known. It is well known that many of the bacteria and certain toxins cause in quantities below the lethal dose a state of malnutrition to which the animal may eventually succumb. The remote effects of the modified venom may be compared to this action of bacteria, ricin, and other toxins.

The time limit of thirty hours, while insufficient to reduce markedly the toxicity of cobra venom, is greater than is necessary to reduce considerably the toxicity of rattlesnake venom. A series of tests was made with the latter venom, which was exposed with eosin for eight hours to sunlight. The result with guinea-pigs of 250 grams each was as follows:

| | | |
|---|---|---|
| 0.002 | gram. | Survived. |
| 0.0032 | “ | “ |
| 0.0048 | “ | “ |
| 0.0064 | “ | Death after 5 days. Slight hæmorrhage. |
| 0.008 | “ | Sick for ½ day; chloroformed after 36 hours; slight hæmorrhage. |

The action of eosin in the light upon Crotalus venom is quite rapid. In eight hours about 9.6m. l. d. and about 16 m. hg. d.[3] of the venom were completely destroyed under the conditions of the experiment.

*Daboia Venom.*—While this venom is rich in hæmolytic and cytolytic principles its chief immediate peculiarity of action results from the thromobokinase which it contains. Whether neurotoxin is present in any but minimal quantities is still undecided. My experiments with eosin and erythrosin were directed against the blood-coagulating constituent. The venom was used in a proportion of 4 parts of 0.2 per cent. and the dyes in 1 part of 0.25 per cent. solutions. The methods employed were identical with those already given. The time of exposure was thirty hours; rabbits weighing 1500 grams were injected intravenously.

TABLE VII.

DABOIA VENOM AND RABBITS.

| Venom solution in c.c. | Control | | Eosin | | Erythrosin | |
|---|---|---|---|---|---|---|
| | Dark | Exposed | Dark | Exposed | Dark | Exposed |
| 0.03 | | | | + 5 days* | | + 10 days* |
| 0.02 | | | | ≀ | | ≀ |
| 0.015 | | | | + 5 days* | | No sympt. |
| 0.01 | | | | ≀ | | " |
| 0.008 | | | | No sympt. | | " |
| 0.006 | | | | " | | " |
| 0.004 | | | | " | | |
| 0.003 | | | | " | | |
| 0.0004 | + 5 min. × | + 2 min. × | + 6 min. × | | +3 min. × | |
| 0.0004 | + 4½ min. × | + 7 min. × | + 5 min. × | | +1½ min. × | |
| 0.0004 | + 7 min. × | + 6 min. × | | | | |

+ = Death. ≀ = Survived after some illness.
× = Intravascular thrombosis.
* = Died of marasmus.

Since these experiments (Table VII) show that the clotting principle of daboia venom is completely destroyed by the fluorescent dyes employed in the experiments, and since they also show that by this treatment the general toxicity of the venom may be considerably reduced, a second series of experiments was made to ascertain roughly the rapidity with which the toxicity is diminished by means of eosin. Rabbits of 1500 grams weight were employed.

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[3] Minimal hæmorrhagic dose.

TABLE VIII.

DABOIA VENOM AND RABBITS.

| Time of exposure | Dose of venom | Results |
|---|---|---|
| 30 minutes | 0.0004 gram. | No symptoms |
| " " | 0.0006 " | + 5 minutes |
| " " | 0.0008 | + 5 minutes |
| 3 hours | 0.0016 " | No symptoms |
| " " | 0.0024 " | + 7 minutes |
| 6 hours | 0.032 " | No symptoms |
| " " | 0.004 " | " " |
| " " | 0.006 " | Sick; survived |
| 8 hours | 0.0016 " | No symptoms |
| " " | 0.0032 " | " " |
| " " | 0.0064 " | " " |
| 12 hours | 0.008 " | No symptoms |
| 30 hours | 0.01 " | No symptoms |

These experiments show that the principle causing intravascular clotting of the blood is quickly and readily destroyed by eosin in the light and that 75 m. l. d. of eosinized venom may not contain one m. l. d. of the thrombokinase. The death of the rabbit, which took place after injection of a large amount of the modified poison was due probably to the combined action of the hæmolytic and neurotoxic residues.

*Action upon the Red Corpuscle-Protecting Principle.*—In a previous publication[4] I described a new property of venom, namely, its capacity to unite with the globulin constituents of blood serum to form an insoluble compound which is precipitated, and with the globin of hæmoglobin which it also renders insoluble in water and weak saline solution. Because of its action upon hæmoglobin strong solutions of cobra venom protect blood corpuscles from water, and even from energetic chemical hæmolysis. Experiments were made with eosin and erythrosin to determine whether this property of cobra venom is destroyed by these agents under the influence of light.

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[4] *Jour. of Exper. Med.*, 1905, vii, 191.

The cobra venom was used in 20 per cent. solution, of which equal parts and 0.1 per cent. solutions of the dyes were mixed. One part of the mixture was exposed to sunlight for 12 hours and the other kept in the dark as a control. The solutions for precipitation consisted of (1) washed rat's corpuscles laked in 9 parts of water and centrifugated to remove the stroma, (2) aqueous solutions of Merck's hæmoglobin, and (3) rat's serum diluted with water. The tests were made with 0.8 c.c. of the solutions enumerated and 0.2 c.c. of the venom-dye mixture. The protective action on the corpuscles was tested by adding 0.3 c.c. of washed rat's corpuscles to 0.6 c.c. of the venom-dye mixture. The results are given in Table IX.

TABLE IX.

| | | Control (no dye) | | Eosin. | | Erythrosin. | |
|---|---|---|---|---|---|---|---|
| c. c. | Control (no venom) | 20% Cobra venom 1 part Distilled water 1 part. | | 20% Cobra Venom 1 part 0.1% Eosin 1 part. | | 20% Cobra venom 1 part 0.1% Erythrosin 1 part. | |
| | | Dark | Exposed | Dark | Exposed | Dark | Exposed |
| lood solution 0.8<br>he fluid tested 0.2 | Clear | Voluminous precipitate (18 hours) | Voluminous precipitate (18 hours) | Voluminous precipitate (18 hours) | Voluminous precipitate (18 hours) | Voluminous precipitate (coarser) (18 hours) | Voluminous precipitate (coarser) (18 hours) |
| % aqueous solu-ion of hæmoglo-in (Merck) 0.8<br>The fluid tested .2 | Clear | Voluminous precipitate (instantan.) | Voluminous precipitate (instantan.) | Voluminous precipitate (instantan.) | Voluminous precipitate (instantan.) | Voluminous precipitate (instantan.) | Voluminous precipitate (instantan.) |
| Rat's serum 0.2<br>$H_2O$ 0.6<br>The fluid tested .2 | Clear | Marked precipitate (18 hours) | Marked precipitate (18 hours) | Marked precipitate (18 hours) | Marked precipitate (18 hours) | Marked precipitate (18 hours) | Marked precipitate (18 hours) |
| Rat's blood corpuscles (washed thrice) 0.03<br>The fluid tested 0.6 | No hæmolysis | Protected | Protected | Protected | Protected | Protected | Protected |

The result is definite. The globulin precipitating and corpuscle-protecting principle of venom does not undergo marked change in sunlight in the presence of eosin and erythrosin after an exposure of 12 hours. If erythrosin in a strength equal to one per cent. of the mixture is present, the venom-hæmoglobin precipitation does not occur, and rat's corpuscles are prevented from protection against hæmolysis. In respect to this action of erythrosin, it should be added that Sacharoff and Sachs state that in solutions of high concentration this dye is itself hæmolytic.

## SUMMARY.

Since the hæmolysins of the several venoms respond differently to photodynamic action, they may be regarded as possessing different chemical constitutions. As regards stability, cobra hæmolysin ranks first, daboia second, and Crotalus third.

The toxicity of all the venoms is more or less diminished by eosin and erythrosin in sunlight. This reduction in toxicity depends upon chemical changes, of more or less profound nature, taking place in certain of the active principles of the venom. The more stabile the predominant active principles the less the reduction in toxicity, and vice versa. Venom-neurotoxins are highly resistant to photodynamic action, venom-hæmolysins are less resistant, while the hæmorrhagin and thrombokinase of Crotalus and daboia venoms exhibit weak powers of resistance to their action. Hence it follows that while cobra venom remained almost unaltered, rattlesnake and daboia venoms were greatly reduced in toxicity when mixed with the fluorescent dyes and exposed to sunlight.

There is an interesting parallel between the action of eosin and erythrosin upon the different venoms and their reactions to other injurious agencies. For example, the hæmolysins of cobra and daboia venoms are more heat resistant than the hæmolysin of Crotalus venom, and the former are less injured by the dyes than the latter. The neurotoxin of the former venoms is also more heat stabile than that of the rattlesnake, and the same relative degree of resistance holds for this substance and the anilines. Just as the hæmorrhagin of rattlesnake venom and the thrombokinase of daboia venom are destroyed by a temperature of 75° C., so are they readily inactivated by the photo dynamic substances employed.

The globulin-precipitating and blood corpuscle-protecting principle of cobra venom is relatively thermostabile and in contradistinction to the immunity-precipitins it is also unaffected by eosin and erythrosin.

This study of the action of photodynamic substances upon snake venoms serves again to bring out the fact of their highly complex nature, and while enlarging somewhat the field in which photodynamic activity is known to operate, it also proves that this form of destructive activity is affected by the same conditions of resistance as confront the action of the usual physical and chemical agents.

## THE LITERATURE ON PHOTODYNAMIC ACTION.

In this brief review only the salient facts can be given. The literature on the biological effects of fluorescence has already grown into a considerable bulk. We shall begin with v. Tappeiner's (1) studies on the chinin derivatives. He proved that the toxic property of chinin resided in the chinolin nucleus and that the toxicity of this body was increased when methoxyl and methylene radicals were present in the side chains. V. Tappeiner further endeavored to increase the toxicity of chinolin by bringing about a further condensation with the benzol nucleus. The proof of the correctness of this view was brought by Grethe (2), who found that the dye phosphin exerted intense toxic action upon paramecium. Raab (3) noted that muriate of acridin in a 1 : 1000 dilution kills paramecium instantly; while weaker solutions acted with disturbing irregularity. The cause of this irregularity was finally traced to the influence of light. Thus in a solution of 1: 20,000 in the dark the protozoa are unaffected, while in diffuse sunlight they are destroyed in 60 minutes, and in direct sunlight in 6 minutes. Raab also ascertained that the action of all protoplasmic poisons is not intensified by light: morphine, phenylchinaldin, and strychnin are not modified in their effects by light. Raab discovered that the increased action of light depends upon fluorescence of the chemical agents. Minute studies have now been made of this property. Raab found that when sunlight passes through a fluorescent solution it is robbed of its power to set up fluorescence in a second solution. No intensification of toxic action is produced in the second solution by the filtered light. Fluorescent light itself is without toxic action upon infusoria; to obtain this effect the living organisms must be immersed in the fluorescent fluid.

We owe to v. Tappeiner the term "photodynamic action," which describes the toxic effects produced by fluorescent chemicals in the light. This activity does not depend upon the absorption of light rays, because some of the most active absorbing agents were shown by v. Tappeiner and Jodlbauer (4,5) not to have their toxicity increased by light. All fluorescent substances would seem to be able to exert photodynamic action; but there is much variation in the intensity of action among the different bodies themselves, and a further variation appears according to the substances—living cells, ferments, toxins—upon which the action is exerted. The relation of degree of fluorescence and intensity of photodynamic action is, according to v. Tappeiner and Jodlbauer (6) a reverse one. Strong solutions may act more energetically than weak ones, but a quantitative comparison shows that, as a rule, the weaker concentrations of the fluorescent body are the more active (7). When certain members of a chemical group do not possess fluorescence they tend to be devoid of photodynamic action. Many salts of fluorescein are photodynamically active; but the non-fluorescent salts—tetranitrofluorescein, phenolphthalin, hydrochinonphthalin—are inactive (8).

Besides the degree of fluorescence and concentration of the chemical body, the intensity of the light plays an effective part. Dreyer (9) found that the infusorian Nassula was killed in 10 seconds under the combined influence of eyrthrosin and Finsen's light, while the Finsen light alone required 9 minutes to produce this effect. Dreyer and Neisser and Halberstaedter (10) viewed the erythrosin merely as a sensitizer and ranked it with neutral red, which they regarded as devoid of fluorescent power. More searching study has brought out the fact of neutral red's possession of feeble fluorescent properties; and v. Tappeiner (11) has been able to show further that sensitization of a photographic plate and photodynamic action are independent powers. Several active sensitizers—bisulphite alizarin blue, diazo black, glycin red, nigrosin, ethylene red—are non-fluorescent and also photodynamically inactive.

In the meantime Straub (12) observed the dissociation of potassium iodide in the light by eosin and chinin, and thus opened the way for the study of photodynamic action as a process of oxidation, proofs of which form of activity were supplied by Edlefsens' (13) studies which were confirmed by Jodlbauer and v. Tappeiner (14, 15). The last-named investigators made a comparative study of the oxidative and biologically injurious properties of photodynamic substances and found that a parallel did not exist. The strongest oxidizing body is not the most effective photodynamic agent. Thus aesculin is an energetic oxidizer but has weak photodynamic power, while the biologically active phenosafranin and azocarmin are incapable even of dissociating iodide of potassium. Jodlbauer and v. Tappeiner also found that photodynamic action may exceptionally take place in fluids from which the free oxygen has been removed. Bie (16) believed the bactericidal action of light not to depend on oxidation. This part of the subject clearly calls for further study pending the setting up of conclusions.

A considerable number of unicellular organisms, toxins, ferments, and antitoxins have been studied in relation to photodynamic activity. The infusoria have been especially studied by Raab (17), v. Tappeiner (18), Jodlbauer and v. Tappeiner (19), and Ledoux-Lebard (20). They all show marked sensitiveness to this form of injury. Jacobson (21) has found that the ciliated tracheal epithelium of the frog is injured by acridin especially in the light. Sachs and Sacharoff (22) and Pfeiffer (23) have described the hæmolytic action of certain fluorescent dyes. Some of these are active in the dark the action being, however, intensified by light (24).

Bacteria are subject to photodynamic action as is shown by the observations of Dreyer (25), Raab, Jodlbauer and v. Tappeiner (26), Huber (27), and Bie (28). Huber found that the filtration of the light through ruby glass prevents the injurious action. Fungi are also susceptible to the action of certain of the anilines. Lichtwitz (29) found that the complements but not the hæmolytic immune bodies of normal and immune serums are destroyed by eosin in the light. And according to Fleischmann (30), eosin and light together so modify precipitin that it no longer causes a precipitate with its corresponding precipitable substance, although it is still capable of entering into chemical union with it.

The ferments show varying degrees of susceptibility to photodynamic activity. Diastase, invertin, papayotin (31), trypsin, and chymosin (32) are affected more or less strongly. Eosin and magdala red are among the most active anilines. Diastase is more resistant than invertin and papayotin. Many fluorescent, photodynamic bodies do not act injuriously upon the ferments. According to Huber (33), rennet is little affected by simple exposure to light, while in the presence of eosin or erythrosin its coagulating action is much delayed. Katalase remains unaffected by any of the fluorescent bodies tested (34).

Bacterial and other toxins succumb to this form of activity (35). Diphtheria toxin is much reduced in power by eosin and light. Other fluorescent anilines are also effective on this poison. V. Tappeiner and Jodlbauer observed that guinea-pigs could be protected from one m. l. d. of diphtheria toxin by previous injection of eosin or methylene blue and daily exposure to the sun. Doses of 3 to 4 m. l. d. were uniformly fatal after this treatment. Huber (36) also found that diphtheria toxin is destroyed by eosin in the light. V. Tappeiner and Jodlbauer noted the modification or destruction of tetanus toxin by certain dyes. They found that after treatment 10 m. l. d. of the toxin did cause local tetanus, while 25 m. l. d. caused fatal tetanus. Huber noted the gradual deterioration of tetanolysin in the light and its rapid destruction in the presence of eosin. Flexner and Noguchi (27) showed that tetanolysin and tetanospasmin are destroyed *in vitro* by eosin, and rats can be protected from fatal tetanus infection by local applications of the dye. Ricin (37) is readily attacked. V. Tappeiner and Jodlbauer found that its agglutinin is totally destroyed by a large number of anilines, while the other toxic constituents are more resistant.

Mammals are not insusceptible to the action of these bodies. Acridin may cause abscesses in rabbits (5), and mice are subject to local necroses if after injection they are exposed to the light. A peculiar effect is the necrosis of the ears of mice, which follows the injection and exposure (38). Exophthalmus has been noted in rats injected with Rose bengale. After injection of eosin, erythrosin, and Rose bengale into the shaved skin of rabbits and exposure to sunlight, an area of necrosis with exfoliation of the tissue develops.

V. Tappeiner and Jesionek (39, 40) have treated with success patients suffering from a variety of parasitic and neoplastic conditions of the skin by applying eosin and exposing the parts to sunlight. Artificial light may be substituted for sunlight and injection into the tissues may sometimes take the place with advantage of superficial application of the dye.

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3. Raab.—Ueber die Wirkung fluorescierender Stoffe auf Infusorien. *Zeitschr. f. Biol.*, 1900, xxxix, 525.

—Weitere Untersuchungen über die Wirkung fluorescierender Stoffe. *Zeitschr. f. Biol.*, 1904, xliv, 16.

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5. Jodlbauer und v. Tappeiner.—Ueber die Wirkung der photodynamischen Stoffe auf Protozoen und Enzyme. *Dtsch. Arch. f. klin. Medicin*, 1904, lxxx, 427.

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7. Straub.—Ueber den Chemismus der Wirkung belichteter Eosinlösung auf oxydable Substanzen. *Arch. f. exper. Pathol. u. Pharmakol.*, 1904, li, 393.

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10. Neisser und Halberstaedter.—Mitteil. über Lichtbehandlung nach Dreyer. *Dtsch. med. Woch.*, 1904, xxx, 265.

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19. See 5.

20. Ledoux-Lebard.—Action de la lumière sur la toxicité de l'eosin et de quelques autres substances pour les paramecies. *Ann. d. l'Institut Pasteur*, 1902, xvi, 586.

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25. See 9.

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29. Lichtwitz.—Ueber die Wirkung fluorescierender Stoffe (des Eosins) auf normale und haemolytische Sera. *Münch. med. Woch.*, 1904, li, 1589.

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33. See 27.

34. Jodlbauer und v. Tappeiner. Ueber die Beteiligung des Sauerstoffs bei der Wirkung fluorescierender Stoffe. *Münch. med. Woch.*, 1904, li, 1139.

35. See 4.

36. See 27.

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38. Jodlbauer und Busck.—Ueber die Wirkung von Fluorescein und Fluorescein-Derivaten im Lichte und im Dunkeln. *Arch. internat. de Pharmac. et Thérap.* 1905, xv, 263.

39. v. Tappeiner und Jesionek.—Therapeutische Versuche mit fluorescierenden Stoffen. *Münch. med. Woch.*, 1903, l, 2042.

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# THE EFFECT OF EOSIN AND ERYTHROSIN UPON THE HÆMOLYTIC POWER OF SAPONIN.

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The chemical action of photodynamic substances is very wide. Not only do they cause alteration of the labile molecules of ferments and toxins, but they are definite and even intense protoplasmic poisons. Their action is, however, not limited to the complex structures and molecules of these bodies, but is capable of being exerted upon much simpler and, chemically speaking, more definite substances.

The more recent investigations of v. Tappeiner,[1] Jodlbauer, Straub,[2] Edlefsen,[3] and Sacharoff and Sachs,[4] all tend to show that fluorescent photodynamic chemicals act by promoting oxidation of the cells, toxins, etc. A wide variety of inorganic and organic well-defined chemical substances was proven to undergo oxidation in the presence of the photodynamic reagents. There were certain exceptions as, for example, dextrose and formaldehyde, which remained unaltered.

In view of these facts, and as a complement to my studies on snake venom, I tested the action of eosin and erythrosin upon the hæmolytic property of saponin. The following tables show that eosin and especially erythrosin act with considerable intensity upon saponin.

The saponin was Merck's, the dyes "eosin rein" and "erythrosin pur." were Grübler's. Saponin was employed in 0.1 per cent., dissolved in 0.9 per cent., salt solution. The dyes were employed in concentrations of from 0.05 per cent. to 2.5 per cent. The mixtures of saponin and dye were divided into two portions, of which one was kept in the dark, the other being exposed to direct sunlight for twelve hours. Control solutions of saponin were treated in the same manner. Two kinds of corpuscles—from the dog and the rat—were used in 3 per cent. suspension. After four hours in the thermostat at 37° C. and eighteen hours at room temperature the readings were made.

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[1] Jodlbauer und v. Tappeiner.—Ueber die Beteiligung des Sauerstoffes be der Wirkung fluorescierender Stoffe. *Deutsch. Arch. f. klin. Medicin*, 1905, lxxxii, 520.

[2] Straub.—Ueber chemische Vorgänge bei der Einwirkung von Licht auf fluorescierende Substanzen und die Bedeutung dieser Vorgänge für die Giftwirkung. *Münch. med. Woch.*, 1904, li, 1093.

[3] Edlefsen—Experimenteller Beitrag zum Studium der fluorescierenden Stoffe. *Münch. med. Woch.*, 1904, li, 1585.

[4] Sacharoff und Sachs.—Ueber die hæmolytische Wirkung der photodynamischen Stoffe. *Münch. med. Woch.*, 1905, lii, 299.

TABLE I.

Saponin 0.1%; fluorescent anilines 0.05%; dog's corpuscles.

| Saponin solution 0.1% | Control | | Eosin 0.05% | | Erythrosin 0.05% | |
|---|---|---|---|---|---|---|
| | Dark | Exposed | Dark | Exposed | Dark | Exposed |
| 1. | C. H. | C. H. | C. H. | C. H. | C. H. | C. H. |
| 0.7 | " | " | " | " | " | " |
| 0.5 | " | " | " | " | " | Much H. |
| 0.4 | " | " | " | " | " | Moderate |
| 0.3 | " | " | " | " | " | Slight H. |
| 0.2 | " | " | " | Much H. | " | None |
| 0.15 | " | " | Much H. | Slight H. | Much H. | " |
| 0.1 | Much H. | Much H. | Moderate | None | Moderate | " |
| 0.07 | Slight H. | Slight H. | Trace | " | Trace | " |
| 0.05 | None | None | None | " | None | " |
| 0.04 | " | " | " | " | " | " |
| 0.03 | " | " | " | " | " | " |
| 0.02 | " | " | " | " | " | " |

TABLE II.

Saponin 0.1%; eosin 0.05%; rat's corpuscles (washed).

| Saponin solution | Control | | Eosin 0.05% | |
|---|---|---|---|---|
| | Dark | Exposed | Dark | Exposed |
| 1. | C. H. | C. H. | C. H. | C. H. |
| 0.7 | " | " | " | " |
| 0.5 | " | " | " | " |
| 0.4 | " | " | " | Almost C. H. |
| 0.3 | " | " | " | Much H. |
| 0.2 | " | " | Much H. | Slight H. |
| 0.15 | " | " | Slight H. | None |
| 0.1 | Almost C. H. | Almost C. H. | Slight H. | " |
| 0.07 | Much H. | Much H. | None | " |
| 0.05 | Slight H. | Slight H. | " | " |
| 0.04 | None | None | " | " |
| 0.03 | " | " | " | " |
| 0.02 | " | " | " | " |

The tables show that saponin alone, in the strength solutions employed, does not deteriorate quickly; and they also show that solutions of this substance have their hæmolytic power considerably reduced when exposed to sunlight in the presence of minute quantities of eosin and erythrosin, and these fluorescent anilines exert a slightly reducing effect upon this property even in the dark. The maximal loss of hæmolytic power equalled in twelve hours about $\frac{1}{4}$ to $\frac{1}{5}$, and in seventy-two hours $\frac{1}{14}$ the total strength.

That concentration of the solutions plays a part in the experiment is indicated by the resistance which a one per cent. solution of saponin exhibited in the presence of eosin in strength of 2.5 per cent. The saponin was not in this case perceptibly reduced in hæmolytic strength after twelve hours' exposure to direct sunlight. If, however, a one per cent. saponin solution is mixed with 0.05 per cent. eosin it is reduced to one-fifth its hæmolytic strength after seventy-two hours' direct exposure to sunlight. V. Tappeiner noted that strong solutions of photodynamic substances may be inactive where weaker ones are effective.

The change in constitution which the saponin undergoes in the presence of eosin and erythrosin has not been established. It would seem, from certain indirect experiments which I have made, not to depend upon the usual splitting of the glucoside. While I did not determine the absolute absence of dextrose in the exposed mixtures, yet I can say that a quantity equal to 0.001 per cent. was not present. Beyond this limit the copper tests which I used were not reliable.

# CONGENITAL CYSTIC KIDNEY AND LIVER WITH FAMILY TENDENCY.

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So much has already been written concerning congenital cystic kidney that an additional communication upon the subject may seem to demand an apology. The author's excuse is a recent opportunity to study two cases in which the lesion was so early that they seem to offer some indication as to the pathogenesis of the condition. The cases were both new-born infants delivered in the obstetrical service of Dr. J. Whitridge Williams in the Johns Hopkins Hospital. Some interest attaches to the fact that they were both children of the same mother, being the first and second children of Mary F., an apparently healthy negro who was sixteen years of age at the time of the delivery of her first child in April, 1904. Her first confinement occurred on April 14, 1904, when a perfectly normal labor resulted in the birth of a male child 44 centimeters in length and weighing 2026 grams. The labor was clinically estimated to have occurred early in the ninth month of pregnancy. The child was placed in the incubator and seemingly did well, gaining in weight after the initial loss, but it died suddenly on the eleventh day.

The second child, a girl, was born on March 21, 1905, eleven and a half months after the birth of the first child. The child was also born prematurely, apparently toward the end of the eighth month of pregnancy. Labor was normal. The child was 42.5 centimeters in length and weighed 2005 grams at birth. It did not do well after birth but lost gradually in weight up to the time of its death when it weighed 1380 grams.

The abbreviated post-mortem protocols of both children will be found appended to this article. For the sake of convenience in description the children will be designated child "A" and child "B."

This family tendency has been previously noted a number of times, not however, in the negro race, and I emphasize it here not only to indicate the part inheritance plays in the condition but also to show that the cases here reported are related in this feature to a considerable group of cases of cystic kidney. Dunger[1] in his admirable review of the subject of congenital cystic kidney has collected eleven cases and added one of his own observation, in which the lesion has occurred in two or more members in the same generation or in two generations of a single family. To these I am able to add several others from literature in addition to the present case.

Schupmann[2] is often credited with having reported the first case showing family tendency, but a reference to his article shows, as Dunger suggests, that he reports not the occurrence of congenital cystic kidney in three children but in the third child of a mother who had given birth to two malformed children previously. This child showed in addition to the kidney lesion hydrocephalus with encephalocœle and a bicornuate uterus and double vagina. Luzzatto[3] includes Adamkiewicz[4] among those who report cases of family tendency, but I have been unable to gain access to his thesis. Virchow[5] in 1855 published the notes of a family in which the mother gave birth successively to three premature children. A post-mortem examination on the third showed bilateral cystic kidneys as the cause of the abdominal swelling. A subsequent child showed a similar condition at the autopsy, and there is little doubt that the same condition existed in the other two children which were not examined. The mother gave birth to four other children, who lived, although one died at the age of ten months as the result of acute hydrocephalus. In 1867 Wolff[6] reported a case of bilateral cystic kidney associated with hydrocephalus in the male child of a woman who had given birth to a female child, previously recorded, with similar lesion of the kidneys eleven months before. The woman had previously borne healthy children. In 1869 Brückner[7] reported two children of the same mother, the third and seventh child, with cystic kidneys and other malformations. The first of these two children showed six fingers on the right hand and seven on the left. The head was set far backward almost on the buttocks and there was also hydrocephalus with hernia of the brain. The second child showed six digits on both hands and both feet and also had hydrocephalus with encephalocœle and atresia of the hymen. In 1894 Singer[8] described a family in which the first (male), fourth (male), seventh (female), tenth (female), and thirteenth (male) children showed the lesion of congenital cystic kidneys, the tumor masses causing great difficulty in delivery, resulting in the death of the children at birth. Only three of these cases were examined post-mortem, but again the presence of tumor masses in the region of the kidney leaves no doubt as to the existence of the lesion in the two unexamined cases. Carbonnel[9] has found the lesion in the kidneys of twins. Höhne[10] in 1896 reported the removal at operation of a cystic right kidney from a girl of 20 years whose mother, dying at the age of 49 years, had shown bilateral cystic kidneys, cystic liver, and cysts in the ovaries. Another child of the same woman had died at the age of 9 weeks of some kidney disease, the author suggests possibly also cystic disease. Steiner[11] in 1899 recorded two interesting families. In the first a man aged 52 died showing bilateral cystic kidneys and cystic liver. His sister also showed the condition of cystic kidneys, and his son of 10 years showed at autopsy a cystic right kidney, while several smaller children were also suspected to have the disease because of albuminuria and palpable nodular kidneys. In the second family the diagnosis of the condition of cystic kidney had been established in a man aged 46, and in a sister aged 38, while it had been demonstrated post-mortem in another sister dying at 42 years of age. In the ten-year-old son of the man a cystic right kidney had also been diagnosed. David and Jacobsohn[12] in 1900 found bilateral cystic kidneys at the autopsy on a woman 50 years of age whose sister had shown post-mortem a similar lesion. The mother of these two women who had died previously presented symptoms suggesting that she had this disease, though it was not actually determined by post-mortem examination. These authors quote Lauenstein's observation of cystic kidney in mother and daughter, as the first to denote a family tendency, but I have been unable to find the original publication. In 1901 Beck[13] reported an operation on a woman of 55 years at which a cystic left kidney was removed. After death 11 days later the other kidney was found extensively diseased. Two sisters of the patient had died previously from the same disease, one at 40 years after an operation, and the other at 54 years. Carrez[14] records the establishment of the clinical diagnosis of bilateral cystic kidney in a woman of 48, as a result of finding at operation a large cystic left kidney in her daughter of 27. Morris[15] records the following two cases. Two children of one mother died of congenital cystic kidney, one at 6.5 months, the other at 3.5 months. Of four other children in the family 3 showed other congenital defects and one was born dead. The father of these children was the only survivor of 13 children, eleven of whom had died at birth and the other at five months. Morris quotes also the report of Bar of three successive children of the same mother, who had died of the disease. Osler[16] in 1902 reported the establishment of the diagnosis of bilateral cystic kidney in a man aged 39, whose mother had shown a similar condition at autopsy. Meyer[17] reported in 1903 the autopsies on two brothers, one 8 months old, the other two years, who had died of diphtheria. In one of these there was present an atrophic cystic kidney on the right side, and in the other a similar lesion on the left. Dunger[18] reports observing a mother aged 64 years and daughter aged 26 years in both of whom bilateral cystic kidney and cystic liver occurred. Both died of cerebral hæmorrhage.

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[1] *Ziegler's Beiträge*, 1904, xxxv, 445.
[2] *Organ f. d. ges. Heilkunde*, 1842, ii, 135.
[3] *La degenerazione cistica dei reni*, Venice, 1900.
[4] Inaugural Dissertation, Berlin, 1843.
[5] *Verh. d. Phys. Med. Gesellsch.*, Würzburg 1855, v, 447.
[6] *Berlin klin. Woch.*, 1867, iv, 480
[7] *Virchow's Archiv*, 1869, xlvi, 503

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[8] Inaugural Dissertation. Greifswald. 1894.

[9] Quoted by Couvelaire, *Annales de gyn. et obst.*, 1899, lii, 453.

[10] *Deutsch. med. Woch.* 1896, xxii, 757.

[11] *Deutsch med. Woch.*, 1899, xxv, 667.

[12] *Charité-Annalen*, 1900, xxv, 16.

[13] *Annals of Surgery*, 1901, xxxiii, 147.

[14] Thesis, Lyon, 1901.

[15] *Surgical Diseases of the Kidney and Ureter*, 1901, i, 656.

In this list it may be noted that a number of the cases showed, in addition to the cystic kidneys, other lesions in the nature of congenital malformations, and such is the frequency of this association that it may be considered almost the rule. The list of associated malformations is long and varied. It is headed apparently by hydrocephalus, although polydactylism is of frequent occurrence. It includes varied lesions to be attributed to imperfections in development in the complicated embryonic folds of the face and perineum, such as hairlip, hypospadias, atresia of the vagina, vesico-rectal fistula, rudimentary condition of the external genitalia. Malformations of the internal genitalia are also noted. In a few cases there have been defects in the cardio-vascular system, among them absence of the ductus Botalli, defects in the interventricular septum and of the aortic valves. In one case there was situs transversus of the thoracic viscera; in another absence of the spleen.

The two cases here reported, however, showed no such gross defects or malformations. Both children though small and prematurely delivered were well-formed externally and their organs were apparently normal, grossly. On microscopical examination, however, there was found in addition to the cystic condition of the kidney, the early stage of a similar cystic condition in the liver, and in one case also in the pancreas. This brings the cases into a fairly large group of cystic kidneys. Lejars[19] collected from literature 60 cases of cystic kidney and of these 28.3 per cent. showed also the cystic lesion in the liver. Luzzatto[20] in 1900 in a larger series of cases found 43 cases or 19.11 per cent. with the associated lesion in the two organs. This association seems important in considering the pathogenesis of the disease and will be discussed later. Turning to the kidneys themselves I shall first describe those of the second child, child "B" as they showed an earlier stage of the lesion than was shown by those of child "A."

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[16] *American Medicine*, 1902, iii, 951.

[17] *Virchow's Archiv*, 1903, clxxiii, 209.

[18] Loc. cit.

[19] Thesis, Paris, 1888.

The kidneys were normally placed in the body cavity and approximately of normal size, perhaps slightly enlarged. The ureters, bladder, urethra, and genitalia were normal. The ureters allowed the passage of a small probe with ease. The kidneys showed nothing abnormal until they were incised, when a considerable amount of urinous fluid poured out and it was found that a number of small cysts had been incised. The larger of these were about 2 or 3 mm. in diameter while there were many others scarcely visible. The cysts appeared to the naked eye to be confined almost exclusively to the medulla of the kidney, with here and there a visible cyst in the cortex. The different medullary pyramids did not appear equally affected, some of them being almost free from visible cavities. The two kidneys were affected and apparently almost equally so.

The tissues were fixed in Zenker's fluid and six sets of serial sections cut in paraffin from various parts of the organs, including a series from the part most affected as well as one from the area apparently freest from lesions. The sections showed that the kidneys in general were well formed. The cortical striation was well marked, with a normal relation between medullary rays and labyrinth, and the cortex was of normal width, measured by the number of rows of glomeruli between the capsule and the medulla which the study of numerous infantile kidneys has shown to be as a general average ten. The glomeruli were well formed and appeared normal with very scattered exceptions. In a few sections were found one or two glomeruli with a dilated capsular space. The tubules of the secreting type were also normal in appearance having a high granular epithelium and a small undilated lumen. In the medullary rays the loops of Henle appeared shorter than normal and in many places did not reach to the boundary zone. There were few cysts in the cortex until one came to a portion of the labyrinth bounding on the medulla. Here there were many elongated and oval cystic cavities lined by a fairly high cubical epithelium. By following out series of sections these could easily be found to be continuous at one end with tubules which entered the medullary rays and followed a course toward the medulla. They often joined other tubules of the collecting type. These tubules were unconnected with the loops of Henle, and were quite evidently collecting tubules, and the cysts thus corresponded to the portions of the tubular system known as the junctional tubules. The connections at the distal end of the cysts were more difficult to trace, but in several places they were found to pass over into tubules with the secreting type of epithelium.

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[20] Loc. cit.

In the medulla the cystic formation reached its maximum. In many places there was scarcely a tubule found with a normal lumen. All were dilated. The cysts were larger than those of the cortex and were variously shaped, tending to be circular and were lined by high cubical epithelium. On the cortical side the cysts opened into several finger-like tubular projections which tapered rather abruptly so that in the boundary zone they were of the width of normal ducts and from here were continued as collecting tubules into the medullary rays. Toward the papilla the cysts became somewhat contracted and in favorable sections could be seen opening freely at the apex of the papilla, into the pelvis. There was no obstruction to the lumen of the ducts at the papilla. In very many places mitotic figures could be found in the lining epithelial cells of the cysts, indicating an active proliferation rather than any compression. There was, however, no evidence of numerical increase in the ducts. Intracystic papillary growths were not made out.

In the kidneys of child "B" the interstitial tissue of the medulla was not increased in amount. The walls of the adjacent cysts lay almost against each other, separated by only a few cells and strands of a rather embryonic connective tissue. The same condition held true for the cortex. In only one or two places was there a slight patchy increase in connective tissue and it was in relation to these patches that the few dilated glomeruli were found.

The sections from the part of the kidney which grossly appeared almost unaffected showed a condition similar to, but of less degree than, that in the other parts. There were almost as many cysts in both cortex and medulla, but they were smaller, resembling more a simple dilatation of the affected tubules.

There is clinical evidence that the kidney functionated, if not normally at least approximately so. The child lived 19 days and apparently passed a normal amount of urine into its napkins. Death was the result of acute gastro-intestinal infection. The sections showed also that the kidney was active and that there was no obstruction to the passage of urine through the tubules. In some of the cysts and tubules there were uric-acid deposits but not in such amount as to cause any apparent obstruction. In some of the tubules of the cortex there were hyaline casts and in one or two places such narrow casts were found projecting into the cortical cysts, demonstrating the continuity of these cysts with the tubules of the secreting type. There was precipitated albumin in some of the glomerular spaces and in the tubules, but no red blood cells were demonstrated.

The kidneys of child "A" were in general like those of child "B." They were about normal in size though the left kidney seemed slightly enlarged. This enlargement was found to be due especially to one fœtal lobule which projected above the others as a rounded pale mass. On section this was found to be much more cystic than the rest of the kidney. The cysts were large, measuring in places 5 mm. in diameter. The tissue between them was pale and fibrous in appearance. In several places in the medulla there were small hæmorrhages, and uric-acid deposits were made out in the form of bright yellow dots and streaks. The microscopical sections showed also a marked similarity to those already described. There was, however, one important difference, and that was a new growth of connective tissue, most marked in the medulla but extending also into the portions of the cortex implicated. This connective tissue was quite richly cellular, but a Mallory connective-tissue stain showed the presence of abundant fibers which had a tendency to take a concentric arrangement about the cystic tubules. In this kidney the greater part of the cystic development was in the medulla, though the cortex was also implicated, and the cortical cysts occupied a position similar to those in the other case, in the portion of the labyrinth nearest the boundary zone. There were, in this kidney also, hæmorrhages into the interstitial tissue of the medulla.

Before treating of the pathogenesis of the renal lesion, I shall describe briefly the lesions of the other organs in so far as they have a bearing on the subject. The liver merits most attention and in the lesions of this organ again child "B" showed an earlier stage than child "A."

Grossly the liver of child "B" appeared slightly smaller than normal for an infant of that age. Superficially it was normal in appearance, the surface being smooth throughout, and the lobulation distinct. The gall bladder and bile ducts were normal. The cut surface, however, had a striking appearance and one that is apparently characteristic of this condition in its early stages. The parenchyma was quite normal in appearance, but everywhere throughout the organ the portal systems were sharply outlined as grayish translucent somewhat branched areas, resembling rather closely an oak leaf in shape. These spaces were evidently much wider than normal. There was a similar increase of connective tissue about the entering portal vessels at the hilum of the organ. The microscopical sections showed that the cause for the increase in the size of the portal spaces was a marked increase in the size and number of the ducts in them, with a slight increase in the connective tissue. This change was uniform throughout the organ. The ducts were lined by a high columnar epithelium, and were free from content other than a finely granular eosin-staining precipitate. These ducts were tortuous, were much branched, and were irregularly dilated into cyst-like spaces. In many places they could be traced to the outer limits of the lobules, where they ended in columns of liver cells of the secreting type. In other places they entered the lobules accompanied by a connective-tissue sheath, and in many places cross-sections of a duct were found, with a similar high epithelium with clear protoplasm, well inside the lobule and even close beside the central vein. The ducts with their cyst-like dilatations in the portal spaces were surrounded by an increased amount of connective tissue which was richly cellular and contained many cells of the lymphoid and plasma-cell type, as well as many eosinophiles. As stated, the condition prevailed throughout the liver, as shown by sections from various parts of the organ. The picture offered by the liver is identical with that drawn to illustrate a case of Borst.[21]

The liver of child "A" was almost identical in gross and microscopical appearances with that of child B, the only difference being a more marked increase in the connective tissue, a difference already noted as existing between the kidneys of the two. The connective tissue in this child's liver was also less cellular and more fibrous and showed a tendency to encapsulate the ducts with concentric layers of fibers.

In the pancreas of child "B" there was found a similar hypertrophic condition of the duct epithelium. The larger ducts showed a much-folded involuted epithelium, and there were more numerous branching ducts in the spaces about the major ducts. Mitotic figures were found in the duct epithelium. The cells of the parenchyma were not so high nor so granular as those of a normal pancreas, and resembled more the duct epithelium. There was some increase in the connective tissue of the organ especially near the larger ducts. It was richly cellular and resembled the tissue about the ducts in the liver. This condition was less marked in the pancreas of child "A."

Section of the duodenum of child "B" showed a definite tendency to hyperplasia of the epithelium, though one hesitates to assign to it any important connection with the lesions of the organs described. The crypts of Lieberkühn were very deep and wide with a high coarse-appearing epithelium of an indifferent type. Some of the crypts were indeed cystic.

In the lungs of both children the terminal bronchi were extremely dilated, but there was no sacculation and the lesion was not apparently of the nature of a congenital bronchiectasis, but of a compensatory nature following incomplete aëration of the lungs.

The other organs showed no lesions which could in any way be interpreted as having a bearing on the disease of the liver and kidneys.

To summarize then, briefly, in two small children of the same parentage there was found a condition of hyperplasia of duct epithelium with a tendency to cyst formation especially marked in the kidneys and the liver, where there was also an increase in the number of ducts, but occurring also to a less degree in the pancreas and possibly in the intestine.

The attempts to explain the pathogenesis of the congenital cystic kidney have been so numerous and so varied that one is inclined to question whether pathologists have been dealing throughout with a single pathological process. Though different processes may be operative in the condition it seems safe to assume that but a single one accounts for the group in which both kidney and liver are affected, and it is to this group that the present case belongs. Any light that may be gained from a study of it may accordingly, it would seem, be applied to the whole group.

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[21] *Festschrift der Phys.-Med. Gesellsch.*, Würzburg, 1899.

If one omits the view that the cysts are formed in the interstitial tissue of the kidney which was early abandoned, and confines himself to those theories which recognize their formation either in the tubules or in the glomeruli, the attempted explanations of the pathogenesis of the condition fall naturally into three or possibly four groups:

(1) that the cysts are the result of obstruction and of retention of secretion ;
(2) that they are of the nature of a neoplasm ;
(3) that they are the result of malformation; and
(4) that the condition lies between the last two, partaking somewhat of the nature of each.

The literature of the subject has been extensively reviewed in many papers, notably those of Luzzatto,[22] Dunger,[23] von Kahlden,[24] and Busse,[25] and I shall make but brief reference to the more important opinions under the several headings of the above classification, acknowledging my indebtedness to these authors, where specific references are not given.

The great exponent of the retention theory was Virchow, and it has probably been the weight of his opinion that has gathered and held so many adherents to this view. There have been many variations in the theory, the contention being as to the nature of the obstruction. The general group of views may, however, be well summarized in the words of Henry Morris[26]: "Regarding as I do the congenital cystic kidney of the fœtus and the new born and the large polycystic kidney of the adult as essentially the same disease, both being due to dilatation, the result of obstruction, one must yet recognize different causes of obstruction in different cases some congenital, some inflammatory, some malformation, some traumatic, some in the kidney, some in the ureter, and some in the urethra. The affection may commence in utero or during the first months of extrauterine life, or in early childhood, or not until middle age, or advanced, or quite old age."

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[22] Loc. cit.
[23] Loc. cit.
[24] *Ziegler's Beiträge*, 1893, iii, 291.
[25] *Virchow's Archiv*, 1904, clxxv, 442.
[26] Loc. cit.

Virchow[27] first advocated the view that uric-acid deposits were the cause of the obstruction but in a later paper[28] advanced the theory that the lesion was the result of a fœtal papillitis or pyelonephritis which caused an increase in the interstitial tissue of the papilla. This in its contraction resulted in the atresia of the papilla and thus in the formation of retention cysts in the tubules. To this view he still adhered in 1892.[29] Frerichs attributed the cysts to retention following obstruction by casts; Bouillard and Lehmann to obstruction by uric acid and calcium salts; Klein, Rosenstein, and for a time Brigidi and Severi, to hæmorrhage from the glomerular tufts with obstruction of the outlet and dilatation of the capsule. Sabourin, Cornil, and Brault attributed the lesion to an inflammatory condition in the kidney resulting, through an interstitial new growth of tissue or cirrhosis, in a constriction of tubules and retention. The retention theory has had many other supporters who see in the cysts of the kidneys of chronic nephritis a lesion analogous to that of the congenital cystic kidney.

In 1875 Sturm[30] expressed the view that the origin of the cysts lay in the epithelium of the convoluted tubules, which at first as the result of a dilatation underwent a simple hypertrophy but later led to the putting out of bud-like processes of an adenomatous nature. By the early fatty degeneration of these constantly growing tumor-like processes, the cysts were formed. In 1876 Mihalkowicz[31] expressed the view that the condition in the kidney was analogous to the cystic disease of ovary, testicle, and breast. Chotinsky[32] could find no evidence that the glomeruli took part in the cystic formation, but found signs, instead, of an active growth of the tubules in outgrowths from the tubules, solid cellular processes, and probably new formation of tubules. He concluded the primary factor was a hyperplasia of the tubules and of the stroma. Lejars[33] described the condition as a persistent congenital lesion, in the great majority of cases of the type of an "Epithelioma Mucoïde." In 1893 Nauwerk and Hufschmid[34] recognized buds from the tubules, intracystic papillary growths, and solid nests of epithelial cells and placed the lesion definitely in the group of adeno-cystomata. Von Kahlden[35] supported this idea, but emphasized farther the coördinate growth of the stroma analogous to that in the adeno-fibromata. He considered the lesion of cystic liver of the same nature. There are numerous other supporters of the theory of active growth of the epithelium of the tubules, among them Hommey, Hausmann, Albert, and Schmitz.

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[27] *Verh. der Gesellsch. f. Geburtshülfe*, Berlin, 1847, ii, 170.

[28] *Verh. der. Phys.-Med. Gesellsch.*, Würzburg, 1855. v, 447.

[29] *Berlin. klin. Woch.*, 1892, xxix, 105.

[30] *Arch. der Heilkunde*, 1875, xvi, 193.

[31] Thesis, Paris, 1876.

[32] Thesis, Bern, 1882.

The frequent association of the congenital cystic kidney with malformation of various parts and organs has led to the idea that it too is to be regarded as a malformation or the result of an error in development. This view gains some support from the extreme complexity of the process of development of the genito-urinary system as a whole. Koster[36] was the first to describe the lesion as an error in development, but he based his theory on a faulty embryology. With the assumption that the tubules of the kidney grew from a separate "anlage" from that of the pelvis and joined it secondarily, he postulated a failure of union between the two parts and a consequent hyperplasia of the epithelium of the tubules with the cystic result. Hildebrand[37] has made use of quite a similar explanation on the basis of the modern dualistic theory of the formation of the kidney, i.e., that glomeruli and convoluted tubules arise from one anlage and secondarily join the collecting tubules which are an outgrowth from the pelvis. Hildebrand postulated a failure of union between these two systems of tubules, with a retention of secretion and cyst-formation in the convoluted portion. Ribbert[38] has made use of the same facts of development to explain the pathology of the condition, but has emphasized, instead of retention of secretion as the causal formation, a wild excessive proliferation of the cells of the tubules which fail to join, particularly of the ampulla of the collecting tubules and the portion of the other section, corresponding to the junctional tubule. He calls attention also to the point that in this active hyperplasia of the epithelium the condition bears some resemblance to a new growth. Meyer[39] and Busse[40] express a similar opinion. Shattock[41] attributed the lesion to the development of retention cysts from portions of the Wolffian body included within the kidney. Bard and Lemoine[42] expressed the opinion that the lesion was due not to a primary epithelial hyperplasia, but to a defective resistance of the walls of the tubules, in particular of the basement membrane, which allowed dilatation under the normal pressure of the secreted fluid they contained.

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[33] Thesis, Paris, 1888.

[34] *Ziegler's Beiträge*, 1893, xii, 1.

[35] *Ziegler's Beiträge*, 1893, xiii, 291.

[36] Quoted by Dunger.

[37] *Archiv für klin. Chirurgie*, 1894, xlviii, 343.

Quite a group of recent writers have taken what may be termed a middle position between the neoplastic and malformation theories. Thus Couvelaire[43] objects to the consideration of the lesion as a tumor of the cystoma or adenocystoma type, and concludes that the process which leads to the cystic transformation consists essentially in an exuberant but general proliferation of the epithelial cells of the excretory ducts, with a correlated reactive growth of the connective tissue. The specific character of the cells is lost. This hyperplasia and perversion in the evolution of the cellular elements take place while the organ is developing and is to be considered as a malformation in that sense. Borst,[44] though retaining the lesion among the cystic adenofibromata, considers the process as an error of development, not in the sense of Ribbert, but in a disturbance of equilibrium between the growth of epithelium and connective tissue, the *sine qua non* for the normal development of an organ. In the cystic liver and kidney, he postulates a failure of the epithelium to develop in a normal way, as the result of a pathological agent, and to possess an increased power of growth. As a result, the connective tissue is invaded. Luzzatto[45] concludes that the pathogenesis of the cystic kidney is not uniform: that in a small number of cases—the shrunken cystic kidneys—the lesion is a result of an interstitial nephritis of fœtal origin; that in the more common type it is to be regarded as either a malformation or a tumor of congenital origin, between which he is unable to draw a sharp line. Still he is inclined to include four congenital cases of his study under the fibrocystadenomata, and a fifth under malformations, due to an excessive development of connective tissue in the organ.

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[38] *Verh. der deutsch. path. Gesellsch.*, 1899, 2te Tagung, 187.

[39] *Virchow's Archiv*, 1903, clxxiii, 209.

[40] Loc. cit.

[41] *Trans. of the Path. Soc.*, London, 1886, xxvii, 287.

[42] *Arch. de méd.*, 1890, ii, 151.

[43] *Annales de gynéc. et d'obstet.*, 1899, lii, 453.

[44] Loc. cit. and Die Lehre von der Geschwülsten, 1902, p. 596.

Dunger[46] recognizes an inflammatory type, but also places the more common in the unsettled province between congenital neoplasms and malformations. He considers the primary factor, however, an error in development, with a secondary proliferation of the epithelial and connective tissues.

In considering these theories from the standpoint of the cases here presented, one notices first that the kidneys offer no evidence whatever in support of the obstruction and retention theory. The urinary path was open throughout from pelvis to urethral orifice. The children lived eleven and nineteen days respectively and passed urine freely. In the kidney the microscopical sections showed the orifices of the ducts of Bellini free and if anything wider than normal. In the kidneys of child "B" there was practically no increase in the interstitial tissue to cause constriction of the tubules. The tubules were in great part free from other than fluid content, and uric-acid deposits and casts, though present, were for the greater part in the least dilated tubules.

While studying these cases, the author has had opportunity to compare the picture afforded by the congenital cystic kidney with that of a congenital hydronephrosis following a stricture of the ureter (Autopsy 2504), in a child which lived but a few days after delivery. The sections of the two lesions were entirely different. In the hydronephrosis there was but moderate dilatation of the ducts of the pyramids, with beginning flattening of the papilla and a beginning proliferation of the interstitial tissue near the mouths of the ducts. There was no indication of active growth of the epithelium. Further, the dilatation of the tubules consequent on the damming back of the urine was noticed not only in the medulla, but also in the cortex, in the convoluted tubules where the lumen was wide and the epithelium somewhat flattened, and even in the glomeruli where the capsules were considerably dilated. In the congenital cystic kidneys, on the other hand, the tubules of the secreting type in the cortex and the glomeruli, with very few exceptions, were entirely unaffected.

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[45] Loc. cit.

[46] Loc. cit.

These cases then give absolutely no support to the theories which refer the lesion to obstruction and retention. There is as little evidence afforded to support the idea of any malformation or error in development of the kidneys in the sense of Hildebrand and Ribbert—that is, of a failure of the two sets of tubules to join with each other. The glomeruli and cortical tubules were well formed and apparently normal in number. The loops of Henle were possibly somewhat shorter and less well developed than in kidneys of an equal age, but this was the only possible deviation from the normal. If there were a failure of union, one would expect a dilatation of the convoluted tubules or glomeruli due to retained secretion. But in these kidneys no such dilatation was found, and, though the cystic formation was diffuse throughout the kidney, there was anatomical and physiological evidence that the urinary path from glomerulus to pelvis was free.

The sole malformation, if it may be called such, was an active proliferation of the epithelium of the collecting ducts of the medulla and of the junctional tubules in the cortex, chiefly in the inner part. Of such active proliferation, there was positive evidence in the numerous mitotic figures found in the epithelial lining of the cysts in child "B." The absence of an increase of connective-tissue growth in the kidney of this child and its presence in that of the other seems evidence also that such sclerosis is a secondary phenomenon. In the kidney I was unable to satisfy myself that there was an excessive formation of tubules. In the liver, on the other hand, where that point is more easily determined from the architecture of the organ, the ducts were not only larger than normal but much more numerous, and in many cases penetrated the lobules almost to the central vein. There seems no question that one must consider the processes in the two organs as essentially the same, and this suggests the possibility that some of the cystic tubules in the labyrinth of the kidney cortex, which clearly showed a connection with the ducts of the medullary rays, but which much less rarely could be found to continue into tubules of the secreting type, were new-formed tubules, analogous to those ducts of the liver which penetrated the lobule. The condition in some particulars thus resembles a neoplasm, yet it is as readily considered a malformation in the sense of an abnormal power of growth of the duct epithelium. If one accepts the Cohnheim theory for the origin of certain tumors in the congenital misplacement of tissues, an error in development, there is no sharp line between the two conditions. However, it seems to the author scarcely appropriate to term the congenital cystic kidney an adenocystoma. Instead of a localized growth more or less sharply outlined from normal tissue, one finds here a diffuse and widespread influence affecting the conducting tubules generally in the kidney, liver, and pancreas, and even possibly in the intestine. The occurrence of multiple tumors in the body is not unusual, but it is to be regarded more or less as a coincidence, and in no group of tumors do we have as constant bilateral occurrence as in congenital cystic kidneys, or as frequent an association as is found between that lesion and the cystic liver. Further, in the several organs the abnormal tubules were directly connected with normal parenchyma, and in the kidney there seems physiological and anatomical evidence that in the cases here reported the cystic tubules carried on their normal function of carrying away the secretion from the parenchyma of the organ. That this functional activity persists for any great length of time I do not maintain. It is readily seen that the increase in the size of the cysts, their encroachment on each other, and the coördinate growth of connective tissue might soon lead to obstruction of a great number of tubules and ducts. This, however, would be a secondary effect.

Primarily, then, one finds evidence of an epithelial hyperplasia in ducts which otherwise appear normal. This leads at first to a general cylindrical increase in the size of the ducts, but gradually there is a greater dilatation at points in the ducts less fixed by the architecture of the organ, and cystic tubules are the result. The expansile force must at first be the normal pressure of the secreted urine, a force which secondarily becomes much greater owing to the mutual pressure of the dilated tubules and consequent obstruction of some of them at various points. It may be objected that a primary weakness of interstitial supporting tissue has not been excluded as the stimulus to the epithelial hyperplasia. To explain why one finds the cystic formation instead of intratubular epithelial growths in case of a primary epithelial hyperplasia, one must assume that the normal urinary pressure is greater than the resistance offered by the supporting kidney tissue. However, that there is any abnormal weakness of this tissue does not necessarily follow, and in fact the specimens offer no evidence of any deficiency in the supporting membrane of the tubules. The tortuosity of certain dilated ducts in the boundary zone, where there appears to be the greatest resistance to expansion, seems an added point in the favor of a primary epithelial process. The position of the lesion in pathological classification would seem to depend on a definition of terms. If it be a neoplasm, it differs from the conditions usually included under that term. If it be a malformation, it is one only in the sense of Couvelaire and Borst—a disproportionate activity in the growth of the epithelium. The frequent occurrence of associated malformations of other parts and organs would appear to indicate that the condition bears a closer relation to malformations than to new growths in the commonly accepted interpretation of the latter term.

From the marked hereditary and family tendency shown in the disease, one is led to search for the etiological factor in the parents. Where the condition has appeared in two generations it has generally been transmitted from father to son and from mother to daughter. Where it appears in several members of one generation, several successive children of either sex may be affected or healthy children may intervene. In one case, Singer's, there was a definite regularity in the appearance of the disease, every third child of thirteen having shown cystic kidneys. The condition then in the parent which determines the lesion in the child is not always operative. What this factor is must for the present be left undetermined.

The abbreviated autopsy protocols of the two cases are as follows:

Autopsy No. 2291. April 28, 1904. Post-mortem examination on Baby F. (child "A"), aged 11 days.

The body is that of a small but well-formed colored male infant, 44 cm. in length. There is some desquamation of the epidermis from the arms and hands, and there are signs of nasal discharge. The abdominal cavity shows a slight excess of clear fluid with marked œdema of the retroperitoneal tissues. The pleural and pericardial cavities are normal.

The heart is of about normal size, and, except for marked distension of the coronary veins, is normal throughout.

The lungs are of pinkish color, deepening to a red in the posterior portion, and crepitate throughout. On section they show marked congestion of the vessels, and the cut surface is moist.

The spleen measures 4 x 2.5 x 15. cm. and is dark red in color. On section the pulp is increased in amount, obscuring the architecture of the organ; Malpighian corpuscles visible.

The liver is about normal in size with smooth regular surface. On section it has a dark purplish color, mottled with areas of light grayish translucent appearance, which are leaf-like in shape. These areas seem firmer than the liver parenchyma. There are scattered throughout the organ also, small light colored areas which are just visible. The limits of the lobules cannot easily be made out. There is an increase in the connective tissue about the larger vessels. The gall bladder and ducts appear normal.

The pancreas, stomach, and intestine show no definite lesions.

The kidneys are described on page 276.

Adrenals, testicles, and epididymes show no lesions.

The lines of ossification in the femur and tibia are sharp, clean cut, and regular

Aorta and branches are normal.

Microscopical examination. With the exception of the organs described no lesions of importance were found.

Liver. See page 278.

Pancreas. The secreting parenchyma is well formed. Many of the cells contain clear spaces and vacuoles. The ducts are prominent in the section, are dilated, are more branched, and seem comparatively more numerous than in other pancreases of this age.

Kidney. See page 277.

Anatomical diagnosis. Congenital cystic kidneys and liver; catarrhal enteritis. Acute splenic tumor.

AUTOPSY 2515. April 10, 1905. Baby F. (child "B"), aged 19 days.

The body is that of an emaciated colored female child, 42.5 cm. in length. There are no external lesions.

The serous cavities show no lesions.

The heart is of about normal size, and the cavities are filled with rather thick unclotted blood. The valves, endocardium, and myocardium are normal.

The left lung is of light pinkish color, is dry on section, and crepitates throughout. The right lung resembles the left.

The spleen is enlarged and measures 5 x 3 x 1.5 cm. On section the pulp, which is of dark brownish red color, seems increased in amount. The Malpighian corpuscles are enlarged.

The liver is small measuring 10 x 5.5 x 3 cm. The surface is smooth and glistening and the organ seems normally formed. The color is a deep brown. On section the brownish surface shows everywhere leaf-like grayish translucent areas, apparently representing enlarged portal spaces. Otherwise the architecture of the organ cannot be made out. The gall bladder contains a small amount of thick greenish bile. The ducts appear normal.

The pancreas appears normal in size and texture, with no increase in its stroma.

The stomach is distended. Its mucosa is swollen, injected, and covered with thick mucus.

The intestine shows a greenish fluid fæcal content, and considerable mucus on its epithelial surface. The mucosa is injected at intervals throughout its length, and is swollen and velvety.

Mesenteric lymph glands are enlarged, opaque whitish in color, and soft and moist to the touch.

The left kidney is slightly enlarged, and measures 5 x 2.7 x 2.5 cm.; the right measures 4.5 x 2.8 x 2.4 cm. For description see page 275.

The adrenals show no lesion. The bladder and genitalia are normal throughout.

The lines of ossification at the lower end of the femur and upper end of the tibia are sharp and clean cut, not thickened or irregular.

MICROSCOPICAL EXAMINATION—Liver. See page 277.

Pancreas. See page 278.

Duodenum. See page 278.

Kidney. See page 275.

The lesions in the other organs are not of interest.

Anatomical diagnosis. Congenital cystic kidney, liver, and pancreas; acute catarrhal gastro-enteritis; acute splenic tumor; acute mesenteric lymphadenitis.

# THE RESUSCITATION OF THE CENTRAL NERVOUS SYSTEM OF MAMMALS.*

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*From the Hull Laboratory of Physiology, the University of Chicago.)*

Introduction.
Technique of the Experiments.
The General Results of Occlusion of the Cerebral Arteries.
The General Phenomena following Restoration of the Cerebral Circulation.
Summary and Conclusion.

## INTRODUCTION.

The literature on anæmia of the central nervous system is extensive. A review of the work on cerebral anæmia up to 1900 is given by Hill.[1] Hayem and Barrier[2] review the work on resuscitation of the isolated brain up to the time of publication of their results. Further summaries of previous work on resuscitation in general are given by Batelli,[3] Prus,[4] and d'Halluin.[5] It does not therefore seem necessary to review the earlier studies. We shall proceed to a description of our own experiments.

A preliminary account of some of the present series of experiments has been given by Drs. Stewart and Guthrie.[6] Our object has been to gain, at first hand, a knowledge of the condition of the anterior part of the cord and of the brain centers during total acute anæmia, and to determine the ultimate limit at, or below, which resuscitation is possible.

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* The experiments described in this paper were begun by Prof. Stewart and Dr. Guthrie, continued by Dr. Guthrie, Mr. Burns, and Mr. Pike, and concluded by Mr. Burns and Mr. Pike. The paper was written largely by Mr. Pike, with tabulations by Prof. Stewart.

[1] Hill, *Philosophical Transactions of the Royal Society*, 1900, 193, B.

[2] Hayem et Barrier, *Archives de physiologie normale et pathologique*, 1887, x, 3e serie, 1.

[3] Batelli, *Comptes rendus de l'Académie des Sciences*, 1900, cxxx, 800; *Journal de Physiologie et Pathologie générale*, 1900, ii, 443.

[4] Prus, *Wiener klinische Wochenschrift*, 1900, xiii, 451, 482; *Archives de médicine experimentale et d' anatomie pathologique*, 1901, xiii, 352.

[5] D'Halluin, *Comptes rendus de la Société de Biologie*, 1904, lvii, 66; *Résurrection du cœur ; la vie du cœur d isolé; le massage du cœur*, 1904, 118; Th. Lille. *La Presse Médicale*, 1904, xii, 345.

[6] Stewart and Guthrie, *Science*, 1905, xxi, 887.

### TECHNIQUE OF THE EXPERIMENTS.

Nearly all the experiments were done on cats. A few of the early experiments were done on dogs. In the earlier experiments in which blood-pressure changes, and the disappearance of reflexes were the only things desired, a tracheal cannula was inserted. In experiments in which it was desirable to keep the animal alive some hours or days after occlusion, a glass tube, narrowed at the end, was inserted through the mouth and between the vocal cords into the larynx after narcosis by inhalation. This latter method of intubation we adopted later as a routine procedure, it being less difficult and requiring less time than tracheotomy. The tube is retained in position by means of a strong cord tied about the lower jaw. Ether, the only anæsthetic used, was administered through the tube. Artificial respiration was accomplished by slipping the free end of the air tube, from a tank of compressed air, with an escape valve in the side, over the end of the glass tube.

Through an incision in the median line in the lower part of the neck, the right innominate and left subclavian arteries were secured. Careful dissection is required to avoid rupturing the pleuræ and the thoracic duct, both of which lie in the field of operation. The truncus thyreo-cervicalis may be mistaken for the vertebral artery.

The manner of origin of the cerebral arteries is of importance in relation to the production of cerebral anæmia. According to Chauveau,[7] the general form of origin of the carotids in vertebrates below man is by the bifurcation of a common trunk (tronc cephalique) arising from the arteria anonyma. (Fig. 1, a.) Quite as often, however, we have found the left carotid arising separately from the arteria anonyma, and the right carotid and right subclavian arising by the bifurcation of a common trunk. This latter form is given as the usual one for the cat by Wilder and Gage,[8] and Reighard and Jennings.[9] (Fig. 1, c.) We have found, on post-mortem examination, a third form of origin, intermediate between the other two, in which both right and left carotids and the right subclavian artery arise from a common point (Fig. 1, b.) In one case, the left subclavian gave off a branch, of uncertain distribution, proximal to the origin of the vertebral.

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[7] Chauveau, *Traité d'anatomie comparée des animaux domestiques*, 3$^e$ ed. par S. Arloing, Paris, 1879.

[8] Wilder and Gage, *Anatomical Technology*, 2d ed., New York, 1886.

[9] Reighard and Jennings, *Anatomy of the Cat*, New York, 1901.

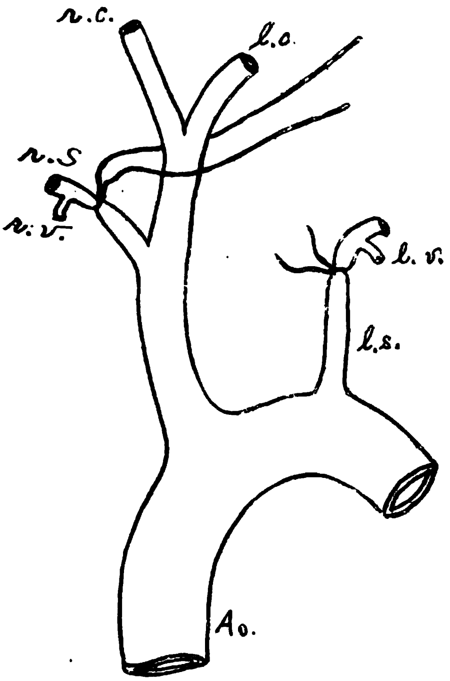

FIG. 1 (*a*). Showing the tronc cephalique of Chauveau.

Ao., aorta ; r.s., right subclavian ; r.v., right vertebral ; r.c., right carotid ; l.c., left carotid ; l.s., left subclavian ; l.v., left vertebral.

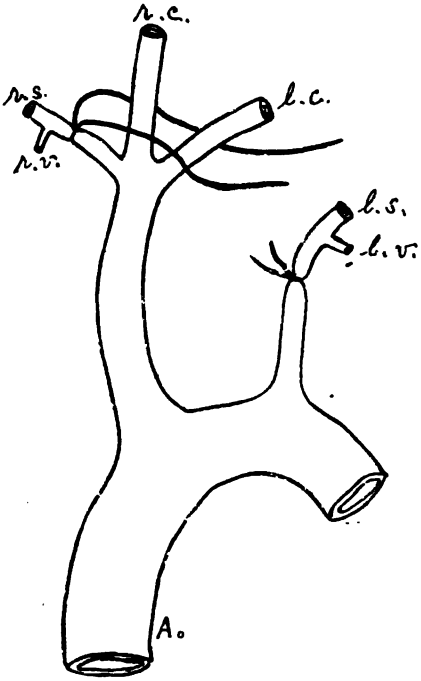

FIG. 1 (*b*). The intermediate type of branching.

Ao., aorta; r.s., right subclavian ; r.v., right vertebral ; r.c., right carotid ; l.c., left carotid ; l.s., left subclavian ; l.v., left vertebral.

Acute temporary cerebral anæmia was produced by passing ligatures around the innominate, and the left subclavian proximal to the origin of the vertebral artery. Traction on the ligatures produced occlusion of the arteries. In case of anomalous origin of the arteries, complete occlusion is assured only when both carotids are clamped separately. In a few experiments the aorta was clamped proximal to the origin of the arteria anonyma.

To keep the animals alive during, and for some time after, the period of occlusion, artificial respiration was employed. The apparatus used for this purpose has proved to be so valuable that we describe it in some detail. An ordinary musician's metronome was fitted with mercury cups and platinum-wire contacts so as to make and break an electric circuit at each complete vibration. The current from one or two batteries was conducted through the metronome and an ordinary telegraph relay and a time marker in circuit with it. This first relay made and broke the circuit through a second relay operated by the electric-light current. A wire soldered to the bar of the second relay operated a valve in the compressed air tube, the distal end of which was connected with the glass tube in the cat's throat.

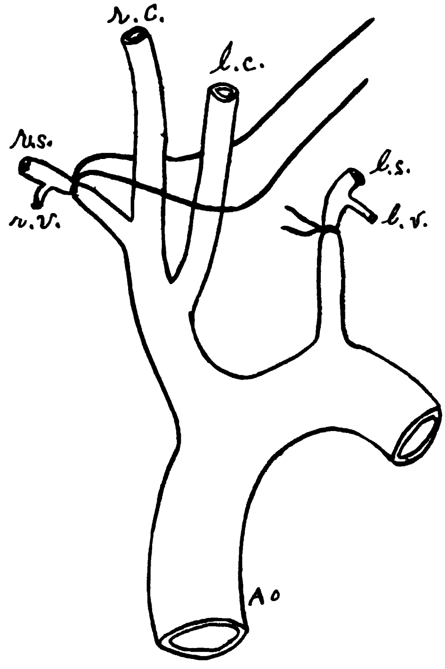

Fig. 1 (c).
**Ao., aorta ; r.s., right subclavian ; r.v., right vertebral; r.c., right carotid; l.c., left carotid; l.s., left subclavian ; l.v., left vertebral.**

Any desired rate of breathing could be obtained by adjusting the metronome.

The blood pressure was always taken from the left carotid artery unless otherwise stated. Sodium-citrate solution was used to prevent coagulation of the blood in the arterial cannula.

When it was desired to keep the animal alive for some time the experiment was done under antiseptic conditions. The neck wound was closed with silk ligatures, sealed with collodion, and bandaged.

Respiratory tracings were made by using the usual tambour connected with the throat tube.

## THE GENERAL RESULTS OF OCCLUSION OF THE CEREBRAL ARTERIES.

*Tests of Occlusion.*—The phenomena of complete occlusion are characteristic and constant. The nose and mucosa of the mouth become white as in death. Respiration ceases, the reflexes disappear, and the pupils dilate completely. The heart is but little affected. Intravenous injection of indigo carmine showed, on post-mortem examination of animals allowed to die without restoration of the cerebral circulation, that anæmia of the brain and medulla oblongata was complete in animals showing these symptoms; that is to say, no trace of the pigment was ever found above the level of the calamus scriptorius. Usually the pigment extended no higher than the third or fourth cervical segment.

Occlusion of the cerebral arteries does not always seem to produce total anæmia, owing, it is to be presumed, to a greater collateral circulation through the spinal arteries. In any event, in a certain number of cases the typical symptoms which we associate with total anæmia are not observed. The persistence of respiration for some time, up to eight minutes, the tardy disappearance of reflexes, and the oozing of a little blood from a nick in the nasal septum distinguish these cases from the first and much the larger group. The disappearance of the reflexes will be considered in detail later in the paper.

*Blood-pressure Changes.*—When the cartoid artery of the animal operated on is connected with a mercury manometer, one of the most striking effects of occlusion of the cerebral arteries is the rapid and great rise in blood pressure. There is at first a rise above the general pressure before occlusion, then a fall, succeeded by a second rise and a second rapid fall to the level which is maintained, with only a very gradual fall, throughout the period of occlusion. The numerical values together with the time relations of each are given in Table I.

TABLE I.

| Number of experiment | Pressure before occlusion. | First rise | | First fall | | Second rise | | Second fall | |
|---|---|---|---|---|---|---|---|---|---|
| | | to | in | to | in | to | in | to | in |
| 1 | 82 mm. Hg. | 102 mm. | 19 sec. | 62 mm. | 32 sec. | 114 mm. | 158 sec. | 40 mm. | 270 sec. |
| 2 | 58 " " | 112 " | 32 " | 46 " | 23 " | 140 " | 115 " | 54 " | 115 " |
| 3 | 126 " " | 156 " | 30 " | 148 " | 60 " | 220 " | 78 " | 100 " | 330 " |
| 4 | 144 " " | 174 " | 9 " | 104 " | 30 " | 190 " | 87 " | 64 " | 05 " |
| 5 | 85 " " | 103 " | 15 " | 70 " | 13 " | 110 " | 45 " | 20 " | 85 " |
| Mean | 99 | 129 | 21 | 98 | 32 | 155 | 97 | 56 | 159 |

Plotting the means of these results, using time as abscissas and pressure as ordinates, we obtain the composite blood-pressure curve shown in Fig. 2.

It will be noticed, on examination of the curve, that (1) the first rise is relatively rapid; (2) the first fall is slightly slower, and sometimes reaches a point below the pressure before occlusion; (3) a second, slower, and usually greater, rise follows, and finally there follows (4) the second fall to a point below the pressure before occlusion, sometimes almost to zero.

The character of the curve is altered when the vagi are divided, the first rise occurring as usual but without the first fall and the second rise.

Section of the cord in the cervical region, as soon after occlusion as possible, causes a prompt and profound fall in pressure.

When both vagi and cord are divided before occlusion a feeble rise of about one centimeter occurs.

To determine the mechanism by which this rise in blood pressure is brought about, we have made a number of experiments. When the abdominal wall is opened, the mesentery exposed and afterward protected from cold, the splanchnic vessels may be observed to contract somewhat, following occlusion. The fall in blood pressure resulting from section of the cord shortly

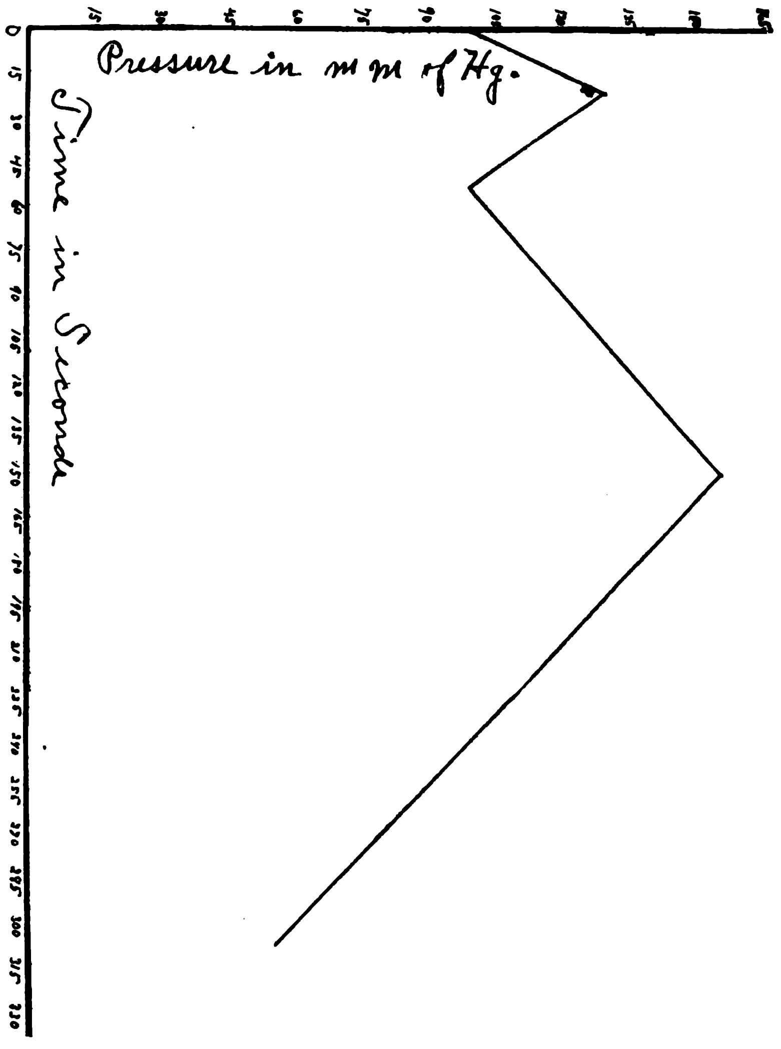

FIG. 2. Composite blood-pressure curve. See Table I.

after occlusion affords further evidence of vaso-constrictor agency in causing the usual rise; and the relatively small rise in blood pressure following occlusion after division of the cord is conclusive proof of the action of the medullary center.

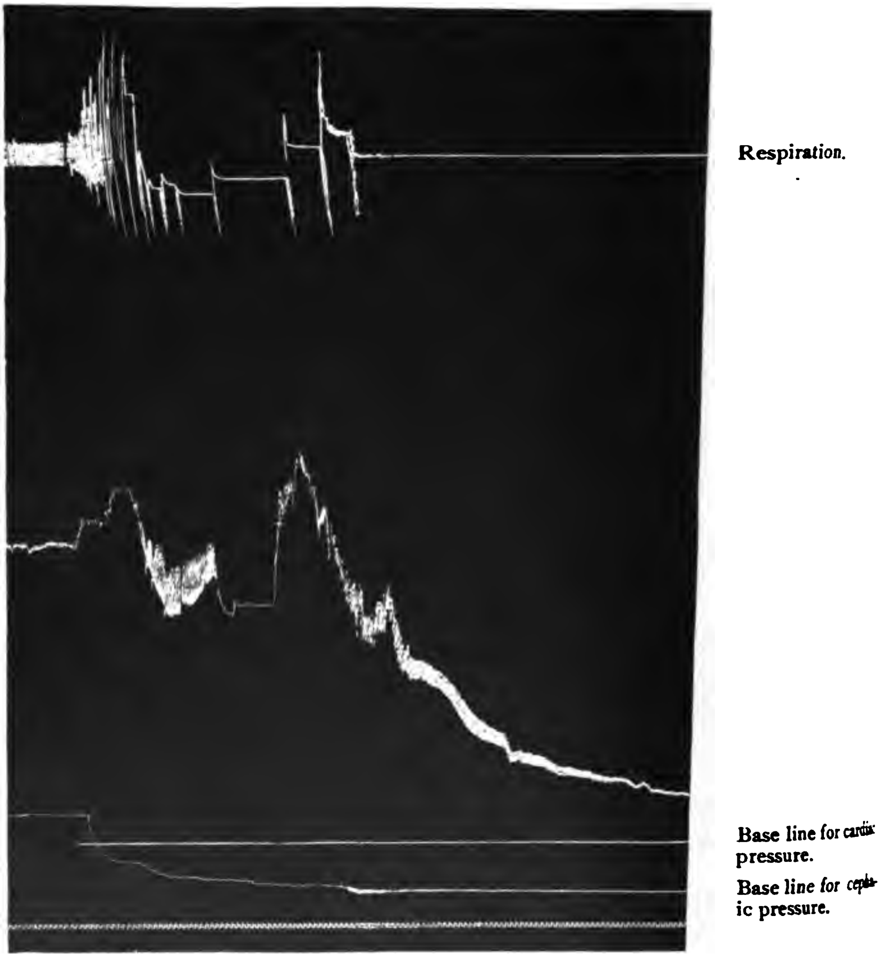

Fig. 3.—Time tracing in seconds. Cannulæ in central and peripheral ends of left carotid. Base line for peripheral (cephalic) pressure first above time tracing; base line for central (cardiac) pressure second above time tracing. Curve of cephalic pressure crosses base line for pressure from central end. Respiration at top, above blood pressure. No artificial respiration. (Expt. 66.)

The cause of the first fall and the second rise we believe to be twofold. (1) When a typical blood-pressure curve (vagi intact) is examined, an increased oscillation of the manometer is observed to begin near the end of the first fall and to continue until near the crest of the second rise (Fig. 3). Pulse counts taken at intervals during occlusion, and a blood-pressure tracing on a rapidly moving drum show that a period of cardiac slowing intervenes here. This inhibition begins at the crest of the first rise, becomes most prominent about the end of the first fall, and then gradually weakens until it disappears entirely at the crest of the second rise.

Bastgen[10], by the injection of oil into the peripheral end of the carotids, and S. Mayer,[11] by the ligation of the cerebral arteries, produced a high blood pressure with slow pulse. Mayer's curves show a distinct vagus inhibition, but without such a marked fall as in our experiments, although he describes a first rise, a fall, a second rise, and a second fall. He ascribes the rise in pressure to (a) a diminished outflow from the heart and (b) an increased inflow from the veins. Bastgen believes that the fall in pressure is due to vagus inhibition, —a suggestion which we adopt as a provisional explanation of the first fall.

(2) Mayer[12] showed that, in Cooper's experiments, the respiratory and vaso-constrictor centers were more tenacious of life than the other cerebral centers. Schiff[13] showed that the accelerator fibers in the vagus resist degenerative changes longer than the inhibitory fibers. Arloing,[14] who repeated Schiff's work, says there are four kinds of fibers in the vagus, one kind being the accelerator fibers, and confirms Schiff's statement as to their greater resistance to degeneration. Hering[15] and more recently Danilewsky[16] have shown that the accelerators retain their vitality longer after the death of the animal (rabbits), than the inhibitory fibers. It is conceivable that the accelerators are more resistant to anæmia than the inhibitory fibers. The second rise in pressure

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[10] Bastgen, Dissertation, Würzburg, 1879.

[11] Mayer, *Sitzungsber. der Kaiser. Akademie der Wissenschaften zu Wien*, 1879, lxxix, 3, Abthl., 87.

[12] Mayer, *Medicinisches Centralblatt*, 1878, xvi, 579

[13] Schiff, *Archiv für die gesammte Physiologie*, 1878, xviii, 172.

[14] Arloing, *Archives de physiologie normale et pathologique*, 1896, viii, 5e serie, 75.

[15] Hering, *Archiv für die gesammte Physiologie*, 1903, xcix, 245.

[16] Danilewsky, *Rousski Vratch* (*Le Médicin russe*), 1904, iii, 641.

is caused by the action of the vaso-constrictor center, and may also be due in part to the action of the accelerators in the vagus. The condition of the vagus center will be considered further in the discussion of the pulse rate.

When the animal has been under anæsthesia for a prolonged period, or when the heart and the respiratory centers have been resuscitated after failure from any cause, or when, for any reason, the vaso-constrictor center in the medulla is in an unstable condition and the Traube-Hering curves appear in the blood-pressure tracing, the first rise of pressure following occlusion and the first fall may be slight or inconsequential, but the slowing of the heart, and the second rise occur as usual. At times, occlusion is followed by the usual first rise, but the first fall in blood pressure is slight, although vagus inhibition may be marked.

After division of the vagi, the first rise in pressure occurs as usual after occlusion, but the period of cardiac slowing and the first fall in pressure are generally absent. We have observed, as did Mayer,[17] that occasional evidences of inhibition are present after section of both vagi. The pathway of such inhibitory influences is unknown to us. It is possible that the inhibition arises from asphyxia from imperfect adjustment of the apparatus. More rarely, the blood-pressure curve following occlusion after section of the vagi approaches the usual form with the vagi intact.

When the cervical cord and the vagi are severed before occlusion, a marked rise of pressure occurs at the instant of division of the cord. A profound fall in pressure follows, then a period of oscillation succeeded by a period of stationary pressure. Occlusion at this time was followed immediately by a slight rise in pressure, approximately 15 mm. of mercury, which persisted throughout the period of cerebral anæmia and fell at the time of release of the cerebral vessels. There was no evidence of inhibition during occlusion. Asphyxiation of the same animal a few minutes later was followed by a curve resembling the usual curve of occlusion, but beginning much more tardily and not reaching as great a height. We are inclined to the opinion that the rise in pressure following occlusion in this experiment was purely a mechanical effect, i.e., due to confining a given volume of blood in a smaller system of vessels.

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[17] Mayer, loc. cit.

Division of the ansa subclavia (Vieusseni), together with all communicating branches from the cervical sympathetic to the vagus below the ganglion nodosum, does not greatly modify the form of the blood-pressure curve following occlusion. In one such experiment, the period of cardiac slowing appeared to be absent, but we have not been able to confirm this result.

In the light of our own results and those of previous observers, we conclude that the vaso-constrictor center in the medulla follows in the wake of the other cephalic centers and loses its power of functioning, but the blood pressure is maintained at a height of 40 to 60 mm. of mercury until the end of occlusion. We decapitated one animal after occlusion. A fall in pressure followed,but, on the intravenous injection of 0.9 per cent. sodium-chloride solution to make good the loss of fluid by hæmorrhage, the pressure rose to 70 mm. of mercury. We conclude that blood pressure is maintained by accessory vaso-motor centers after the medullary center loses its power of functioning.

*The Pulse Rate.*—The period of vagus inhibition has been mentioned in connection with the blood-pressure changes during occlusion. Reference to the tables will show that, compared with the rate before occlusion, there may be an acceleration or a diminution immediately after occulsion. From the beginning of the period of occlusion on to its close there is a well-defined pulse cycle. The rate immediately after occlusion may be increased for twenty to sixty seconds but this is not a constant occurrence. The period of vagus inhibition follows and with it are associated the secondary respiratory gasps, after which the inhibition gradually weakens and ceases entirely, simultaneously with the gasps, both inhibitory and respiratory centers succumbing to the anæmia at approximately the same time. (Experiment 3, Table III.)[18]. The pulse rate then increases to a maximum after which, if the period of occlusion be sufficiently prolonged, it becomes slower until the moment of restoration of the

[18] Tables II and III are given at the end of the paper.

cerebral centers. Asphyxia, after the vagus loses its power of functioning, produces distinct cardiac slowing. This asphyxial slowing must therefore be susceptible of production without the agency of the vagus center. The correctness of this view is shown beyond all doubt by the fact that asphyxia of the animal after section of the cord and both vagi as described in the previous section, produces marked cardiac slowing (Fig. 4).

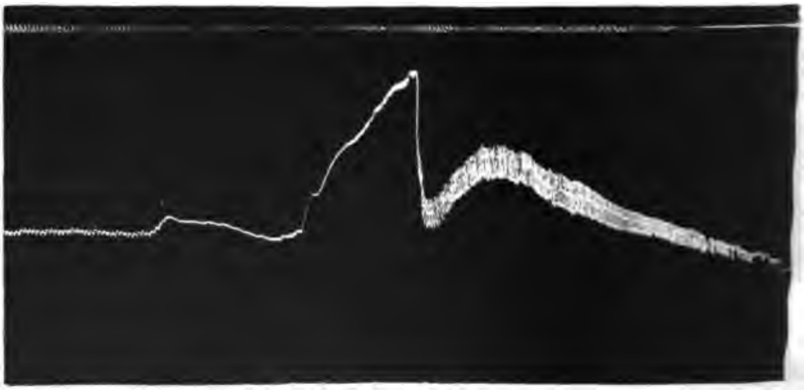

**Fig. 4.—Asphyxial rise of pressure after section of vagi and spinal cord. Asphyxia began at signal. Time trace in seconds. Note cardiac slowing. (Expt. 83.)**

*Respiration* —Respiratory movements continue as usual for ten to one hundred and sixty-five seconds, when they cease. After a pause of thirty seconds to two minutes, a secondary series of gasps of the Cheyne-Stokes type occurs (Fig. 3). There may be only one such gasp, or they may continue throughout a short period of occlusion (eight minutes). Hill[19] thinks that a certain arterial pressure in the respiratory center is necessary to induce respiration. On this point we have no direct evidence. We have taken blood-pressure tracings from the cerebral end of the carotid during the period of occlusion (Fig. 3) and have found in each case a prompt fall to zero after occlusion. When blood pressure and respiratory tracings are made in the same experiment, it is found that the Cheyne-Stokes respiration is coincident with the second rise of the blood-pressure curve. We have thought it possible that some blood might reach the respiratory center through the spinal artery during the period of high blood pressure, but be insufficient in quantity to maintain the vitality of the vaso-constrictor and the respiratory centers; and that as the pressure fell, all blood disappeared from the medullary centers. This supposition accords with Hill's view, but we have no experimental proof to substantiate it.

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[19] Hill, *Cerebral Circulation*, p. 132.

The Cheyne-Stokes-like respiration is independent of artificial respiration, occurring alike in its presence or its absence with vagi divided or intact. The exciting cause of the Cheyne-Stokes group then must at this time be independent of any impulses communicated to the respiratory center from the lungs. Nor does the oxygenation of the blood in the trunk affect the vitality of the respiratory center during total anæmia, the gasps ceasing as soon with artificial respiration as without it. It should be noted also that in only one of our experiments was the Cheyne-Stokes respiration absent, the gasps being present even in experiments in which the chest wall had been opened before occlusion. As has been stated, the vagus and the respiratory centers become functionally inactive at approximately the same time. No further respiratory movements occur until after the restoration of the cerebral circulation.

*The Disappearance of Reflexes.* — Observations have been made on the time of disappearance of the light, lid, corneal, and dilator reflexes. In case of good occlusion, the light, lid and corneal reflexes vanish so quickly as to make an accurate time record almost impossible. In one experiment these reflexes persisted for twenty-seven seconds, but respiratory gasps persisted throughout the period of occlusion, which was ten minutes. We doubt the totality of the anæmia in this and similar cases. In nearly every experiment before occluding the animal was allowed to come out from the influence of the anæsthetic until the pupils were well contracted, and the corneal reflex present in some degree. Often the eyelid closes on being touched directly, while touching the cornea gives no response.

The pupillo-dilator phenomenon is more persistent than the others of this group and is sometimes more persistent in one eye than in the other, but this is probably due to injury of the left ansa subclavia, as will be pointed out later. Ten seconds to a minute may elapse after occlusion before the pupil begins to dilate. The movement is rapid, four or five seconds being sufficient time to allow of maximal dilation. Occasionally the pupils dilate as usual, contract as the secondary respiratory gasps begin, and again dilate to the maximum before the cessation of these gasps. In no case have we observed the contracted state of the pupils persisting longer than the respiration, a point on which we differ from Cyon.[20] We are unable to see the reason for the persistence of the reflexes for such long periods as he gives, and we suggest that cerebral anæmia in his experiments may have been less complete than our own.

Soon after the dilation of the pupils, the cornea becomes lax, then sunken and furrowed, indicating a reduced intra-ocular pressure due, possibly, to a number of factors, e. g.: (1) a decrease in blood pressure in the vessels in and around the eye; (2) the escape of fluid from the anterior chamber of the eye; (3) loss of tone of contractile tissues of the eye and (4) loss of fluid from both chambers of the eye by diffusion and filtration into the contiguous anæmic areas.

The time of the disappearance of the various reflexes, and of their return after the restoration of the cerebral circulation is given in Table II.

Placing the hand on the chest causes a fall in blood pressure, with a rise when the hand is removed. Striking the abdomen causes a fall in pressure. Pinching the hind-feet with forceps causes a rise in pressure. Similar pinching of the fore-feet gives no result. Stimulation of the central end of the left cut vagus, the right being intact, twenty minutes after the beginning of the occlusion caused a rise in blood pressure. Cutting the right vagus of the same animal two minutes later also caused a rise in blood pressure Stimulation of the central ends of the vagi, both divided, thirty-four minutes after the beginning of anæmia caused no change in the blood pressure. A stronger current applied to the tissues of the neck caused a rise.

*Muscular Movements.*—There is usually a sudden stiffening of all the muscles, following occlusion, with violent movements of the fore- and hind-limbs and tail. Micturtion and defecation may occur. Then the fore-limb movements cease, shortly afterward the hind-limbs relax, and the whole animal lies limp and quiet until after the restoration of the cerebral circulation. The intravenous injection of strychnine sulphate during the period of occlusion is followed by the usual spasms of the abdominal and hind-limb muscles, but no effect, except a possible slight exaggeration of the reflexes is observed in the fore-limbs, and only when the strychnine is injected immediately after occlusion and before the period of high blood pressure. The anterior part of the cat may be completely relaxed and inert while the posterior half may show violent strychnine spasms as in the normal cat. The blood pressure rises after the injection of strychnine. The early exaggeration of the anterior reflexes again suggests the possibility of an increased flow of blood through the anastomotic channels during the period of high blood pressure, but the subsequent complete relaxation of all anterior muscles, even when twice the minimum fatal dose of strychnine is injected, indicates that the anæmia of the anterior part of the cord is very complete.

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[20] Cyon, *Comptes rendus de la Soc. de Biol.*, 1900, lii, 372.

The picture presented at the close of a typical occlusion is an animal with widely dilated pupils, lax and sunken cornea, motionless eyelids, no tear secretion, no saliva, bloodless mucosa of the nose and mouth, relaxed muscles, no voluntary respiratory movements, low blood pressure, with a heart becoming slow and weak, perhaps stopping if the occlusion be long continued. The whole sequence of events resembles in a general way those found in death by hæmorrhage, as described by Hayem.[21]

## THE GENERAL PHENOMENA FOLLOWING RESTORATION OF THE CEREBRAL CIRCULATION.

*Blood-Pressure Changes.*—The empty cerebral arteries fill rapidly after removal of the ligatures. The mucosa of the nose and mouth lose their pallor and the blood flows freely from a nick in the nasal septum. The manometer may show a fall,

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[21] Hayem, *Archives de physiologie normale et pathologique*, 1888, i, 5e serie, 103.

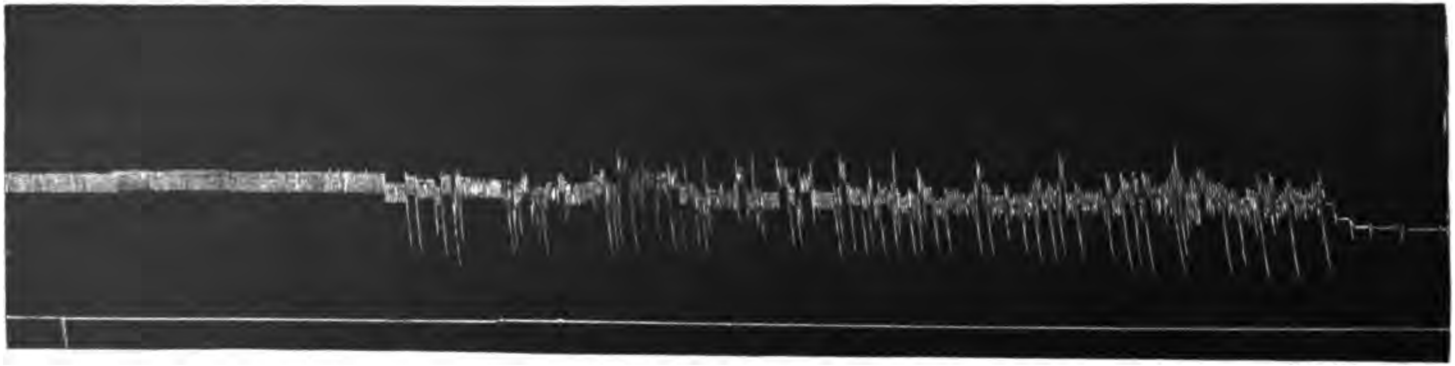

Fig. 5.—Return of the respiration. The cerebral circulation was restored at signal. Note that the first gasp, as indicated by the greater excursion of the writing point of the tambour, was a strong one. Shorter excursions of lever at right of tracing mark the beginning of natural respiration after cessation of artificial respiration. (Expt. 77.)

sometimes very marked. The pressure may even fall to zero, the heart stop, and death result. Examination of the heart, by opening the thorax immediately, may show a dilated right heart with the left ventricle in spasm.

In most cases, a slow rise in pressure succeeds the fall, continuing until the pressure before occlusion is approached or reached. Traube-Hering curves and other irregularities appear in the blood-pressure tracing. Gradually the center apparently becomes more stable in function and the irregularities disappear. Stimulation of the sciatic nerve is followed by the usual rise in pressure at this period, although it may not cause a rise in those cases where the pressure is very low and which terminate fatally.

*Pulse Rate.*—The rate before release of the cerebral arteries may increase after the restoration of the cerebral circulation, to diminish as the time of the first respiratory gasp approaches (Experiment 10, Table III), again increase as respiration becomes established, perhaps become very rapid, and finally, after some hours or days, return to a normal rate. The tables show pulse counts beginning at the time of release of the cerebral arteries, and continuing for five days thereafter. After prolonged periods of anæmia, the heart may gradually stop.

*Respiration.*—The disappearance and the return of the respiration are more definitely marked and constant phenomena than the disappearance and return of the eye reflexes.

The animal lies motionless after occlusion, with the blood pressure gradually rising. The first sign of returning vitality in the respiratory center is a strong gasp (Fig. 5) followed by another and another, the rate being about four a minute at the outset, and gradually increasing until spontaneous respiration is established. The respiratory rate is, at first, usually much slower than that of a normal cat, and hours or even days may elapse before the normal rhythm is reëstablished. After prolonged periods of anæmia, e.g., eighty-one minutes, the respiratory gasps may not occur after the release of the cerebral arteries. An occlusion of twenty-eight minutes was followed by a first gasp forty minutes after release. The gasps continued fifteen minutes, six to eight per minute, and then ceased. Artificial respiration was continued, but dilation of the pupils and, later, failure of the heart followed. Apnœa is frequently met with following long occlusions. Respiratory gasps occur, become almost frequent enough for spontaneous respiration, and suddenly cease. Interruption of artificial respiration at such a time is followed by dilation of the pupils and collapse. If the blood is properly oxygenated, respiratory movements may begin again in twenty to sixty minutes. Cessation of artificial respiration after fifteen minutes of apnœa was followed by fifty seconds of quiet, then a spasm of the fore-legs and chest muscles, a gasp, relaxation of the spasm, and slow spontaneous respiration. In one experiment, apnœa continued for two hours.

It is worthy of note, that, in one experiment in which it was necessary to open the chest walls and massage the heart directly, ten minutes after it had ceased to beat, deep but ineffectual spontaneous respiratory gasps occurred as the blood pressure rose, each of which was followed by a sudden fall of pressure. It appears that, in this condition of the animal, a certain pressure in the respiratory center is necessary, as Hill states, to provoke respiration.

The connection between the vagus and the respiratory centers after occlusion has been noted. Pulse counts indicate a similar connection after restoration of the cerebral circulation, a distinct cardiac slowing, as has been noted, often occurring at the time of the first respiratory gasp. A very early sign of returning function occurring before respiration or contraction of the pupil is the fibrillating movements of the tongue, closely resembling fibrillation of the heart. It is observed after occlusions of ten minutes or more, and continues until the other reflexes are well established. A closely related phenomenon is the twitching of the skin of various parts of the body, but especially of the shoulders and thighs. It generally appears a little later than the fibrillating movements of the tongue, and lasts about as long.

*Reflexes.*—The light, lid, and corneal reflexes are inconstant. The lid and corneal reflexes may return relatively soon after release of the cerebral arteries, while the light reflex may not be capable of demonstration until two or three days later. None of them returns after long periods of occlusion, e.g., sixty minutes. The leg reflexes are considered later. The pupillo-dilator action presents some peculiarities. We have never made an experiment in which the pupils remained contracted after failure of respiration. In cases of paralysis of the respiratory center during anæsthesia, the pupil first dilated to the maximum, the animal perhaps made two or three expulsive gasps after which the respiration failed. Respiration was always reëstablished before the pupil contracted much, but the pupil sometimes began to contract before respiration was established. After release of the cerebral arteries gasping respiratory movements appear before complete contraction of the pupil, but the pupil may be narrowly contracted before respiration is fully established, and usually remains contracted in apnœa of brief duration. It is difficult to differentiate in this condition between the action of the cerebral center, and the action of the cervical sympathetic which, as Tuckett[22] has shown, will withstand vastly longer periods of anæmia than the cerebral centers. Two and three days after occlusion may find the pupils widely dilated, while respiration proceeds without difficulty.

Intra-ocular pressure is reëstablished before, coincidently with, or more rarely after, the contraction of the pupil.

The picture presented at this stage is one in which the natural pink has replaced the deathly pallor of anæmia, the tongue is fibrillating actively, the skin twitching, pupils contracted, and respiration spontaneous but slow. The corneal, lid, and light reflexes may be present. If the period of anæmia is prolonged, the cornea may be lax and dry. The tears gather in the outer corners of the eyes and the eyelid trembles preparatory to closing completely and moistening the dull, dry cornea with the secretion which is in waiting. The cat may appear as if asleep with its eyes open, but the picture is soon to change.

*The Onset of the Convulsions.*—Convulsions, varying in severity from occasional twitchings of the limbs to extreme opsisthotonus and violent struggles, of tonic or clonic type, sometimes begin before the full return of the reflexes. Occasionally the cat may be quiet for one to three hours before the convulsions begin. The head may be drawn back in extreme opisthotonus, or may be bent downward with the back convex. There may be tonic spasm of the fore-limbs and clonic spasms of the hind-limbs. Again, there may be violent clonic spasms in which the animal flings itself about from one side of the room to the other. The cat may lie quietly on its side, the head in moderate opisthotonus, the fore-legs outstretched in tonic extensor spasms with alternate protrusion and retraction of the claws. If the head is gently pushed forward until it is in line with the long axis of the body, the flexor muscles then go into spasm so that the tip of the nose and the hind-feet are approximated. Soon, however, the extensor muscles overcome the flexors, and opisthotonus returns. The convulsions may follow in such rapid succession as to make it difficult to distinguish any interval between successive seizures. At other times intervals of five to twenty minutes elapse between any two convulsions. The pupils dilate widely during the spasms. Respiration may be impeded during the spasms, and rapid during the intervals. Attempts at vocalization are confined, as a rule, to the intervals between convulsions. Stroking the fur, clapping the hands together near the animal's ears, walking across the floor, passing a thermometer bulb into the rectum, and even blowing gently across the fur may throw the animal into spasms. The period of frequent and violent spasms lasts two or three hours to as many days.

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[22] Tuckett, *Journal of Physiology*, 1905, xxxiii, 77.

Micturition and defecation occur as usual during this period. In all cases in which urine voided after occlusion has been examined, it has contained a substance which, on boiling, strongly reduces Fehling's solution. Polariscopic examination shows it to be dextro-rotatory. In one experiment, three samples of urine voided two hours and twenty minutes, three hours and thirty minutes, and twenty hours respectively, after the beginning of occlusion showed the highest percentage of reducing substance in the second sample. A very slight fermentation occurs with yeast but nothing at all proportional to the amount of reducing substance present. The nature and cause of this condition are unknown to us.

The temperature is often subnormal, and may not rise to normal for several hours, or even days. It has appeared to us at times that a high temperature may increase the severity of the spasms, while a reduction of the temperature by putting the cat in the ice-box may reduce their severity, but of this we are not sure. The presence of a cloth covering in which the animal can entangle itself increases the severity of the spasms, as the struggles continue until the covering is thrown off. The most successful treatment consisted in keeping the animal as quiet as possible, covering it when practicable to do so without increasing the spasms, but relying mainly upon perfect quiet and freedom from jar to eliminate the spasms. Ether and chloral will stop the spasms, but may stop respiration and heart-beat also. Seizing the feet and stretching the limbs subdue the spasms during the several seconds that the limbs are held.

Severe convulsions may terminate in death twelve to thirty hours after their onset. The direct cause of death is unknown. The condition of the brain and anterior part of the cord at the end of occlusion is such as to allow of no reflexes. Fifty-two minutes after release, following an occlusion of fourteen minutes, there was no response of the anterior part of the cord to the intravenous injection of strychnine. There was no response twenty minutes after release following an occlusion of seven minutes. Strychnine at this time appeared to retard rather than accelerate the recovery of function by the anterior part of the cord. Severe strychnine spasms of the posterior half of the body followed the subcutaneous injection of strychnine, after the return of respiration and eye reflexes, thirty-three minutes after release from an eight-minute occlusion, but the anterior half of the body was relaxed. Seven hours after release from an occlusion of eight minutes, the anterior part of the cord had regained its irritability to strychnine. General spasms appeared, stronger in the hind-limbs than in the fore. On increasing the strychnine, the fore-limbs relaxed before death while the hind-limbs were still in spasm. Another case presents some unusual features which we give in detail.

Experiment 102. Seven and one-half minutes of anæmia in the morning, with recovery, was followed by 21 minutes of anæmia in the afternoon. All reflexes returned after the second period of anæmia. Respiratory gasps began 16 minutes after release, continued 47 minutes, when a period of apnoea supervened. Artificial respiration was discontinued after 15 minutes of apnoea. The cat lay quiet 50 seconds; spasm of thorax muscles and fore-limbs, then a gasp and relaxation of spasm, and slow, spontaneous respiration followed. Left half of cord hemisected 95 minutes after release. Cat was allowed to remain undisturbed until 5 hours after occlusion. At 8 P. M., cat lay on its side, moderate opisthotonus, strong tonic spasm of fore-legs, hind-legs relaxed. Moving left hind-leg caused convulsive twitchings of both hind-legs, and mild tonic extensor spasm of right hind-leg; pupils normal; left nictitating membrane prominent. Strychnine sulphate (0.6 mg.) subcutaneously. Prick of the needle increased opisthotonus; reflexes increased, general spasms appeared, became extremely violent, and resulted in failure of respiration one hour after injection of strychnine. Fore-legs relaxed 9 minutes before respiratory failure, while spasm of the hind-legs continued up to that time. Heart stopped, all muscles relaxed, whole animal limp. Intubation and artificial respiration. Thorax opened, direct massage of heart. Heart beating regularly at 9 20, 13 minutes after its failure. At 10.40 heart was beating regularly; muscle tonus returned so much that hind-legs and tail moved spontaneously. No reflexes were obtainable anterior to posterior margin of the shoulder. Artificial respiration stopped. Auricular beats of heart observed 18 minutes afterward; no later observations were made.

The first reflex response to pulling or pinching in the front legs occurs in the leg of the same side. Later, striking one fore-leg causes reflex movements of both.

Experiment 28. Occlusion of 10 minutes, 23½ minutes after release, reflex contraction of fore-leg occurs, but only on side struck. Twelve minutes later there is crossing of reflexes, and both fore-legs contract when one is struck.

Experiment 29. Occlusion of 20 minutes; 32 minutes after release, reflex contraction of fore-leg struck, and slight contraction of opposite fore-leg. In 4 minutes more, crossing of reflexes is distinct, and both fore-legs contract when one is struck.

Experiment 32. Occlusion of 50 minutes (aorta clamped); 10 minutes after release, twitching movements of moustache. In 3 minutes, twitching movements of throat muscles over larynx where exposed in neck wound. In 33½ minutes, twitching of muscles over upper ribs in wound. In 55½ minutes twitching of skin of neck. In 67½ minutes respiratory gasps begin, involving muscles in front of neck between the shoulders, gradually spreading from below upward in the neck, including the shoulders; gasps about three per minute. 73 minutes, strong twitching of right shoulder. 77 minutes, slight spasms of shoulders and fore-limbs. Marked reflex on striking fore-limb with strong crossed reflex of the other. 83 minutes, strong general spasm of anterior end of body. Then a strong gasp, the lower jaw coming down, breast and shoulders coming up.

It is possible that the lower portion of the cervical cord suffers less injury than the upper portion, the medulla, and the brain.

Experiment 18. Anæmia 20 minutes; 110 minutes after release, stimulation of cortex caused jaw movements of opposite side. No other movements except perhaps slight movements of opposite fore-limb. The opening of the cranial cavity was accompanied by some hæmorrhage, but as soon as this was done, deep, regular, spontaneous respiratory movements began, involving head, neck, and thorax down to the diaphragm. It was not observed whether the diaphragm participated in these movements or not. These movements were much more effective for respiration than previous respiratory movements. It is impossible to say whether trephining or hæmorrhage, by reducing intra-cranial pressure, removed the previous cause of the inhibition of the respiratory centers.

*Transection of the Cord.*—Division of the cord in the upper dorsal region (III to VI dorsal vertebræ), if done before fairly complete recovery of the cerebral centers, is followed by collapse, dilation of the pupils, cessation of respiration, cardiac failure, and death. The integrity of the spinal centers is necessary for the resuscitation of the cerebral centers. Intravenous injection of warm 0.9 per cent. salt solution has proved useless in averting or remedying such conditions.

Division of the cord after the spasms have become well established is followed by transient shock, recovery, and cessation of the spasms in the hind-limbs. Hemisection of the cord is followed by cessation of spasms in the hind-leg of the same side.

Experiment 50. Occlusion of 15 minutes partial, 40 minutes total, anæmia of brain. In 84½ minutes from release, strong clawing movements of hind-legs, with contraction of muscles of abdomen and thorax, up to neck, during spasms of clawing. Division of spinal cord just below last rib three hours after release. Spontaneous clawing movements of hind-limbs disappear, and do not return. Fore-limbs contract much better on pinching than before division of cord, but reflexes of hind-limbs are much less marked.

Experiments 42 and 43. Occlusion of 60 minutes. Tried to subdue spasms by ether. Death from ether 16 hours after release. Urine collected and kept in an ice-box until next day, and then injected into a half-grown cat. Needle inserted over the liver. Seven hours after, convulsions appeared in second cat; pupils dilated, hair on back and tail, erect. Spasms pass off in 30 minutes. More spasms on second day after injection. Dead on third day. We have neither carefully controlled nor have we confirmed this experiment.

Krainsky[23] has suggested that the presence of certain waste products, e.g., ammonium carbonate, in the blood may cause spasms. Donath[24] has found cholin in the blood of epileptics. More recently, Allen,[25] using a new method of testing for cholin has failed to find it in certain nervous conditions as reported by other investigators. We have made no chemical analyses of blood or urine beyond those above mentioned. The spasms produced in the kitten by injection of urine from a cat in convulsions might be held to indicate the presence of some circulating toxin, but we do not know that the kitten had not previously suffered from spasms due to another cause. The experiments on section of the spinal cord show that whatever toxins there may be in the blood have no effect on the part of the cord not previously subjected to anæmia.

*Other Structures Affected by Anæmia.*—The lack of salivary and lachrymal secretion during occlusion, and the reappearance of tears have been noted above.

Experiment 32. Occlusion, 50 minutes. No return of lid or light reflexes First gasp 67 minutes after release. Stimulation of chorda tympani 3 hours 8 minutes after release gave some secretion.

Experiment 21. Occlusion of 6¼ minutes. Heart stopped 11 minutes, and again 8 minutes. Stimulation of chorda tympani 2 hours and 17 minutes after starting heart gave good flow of saliva.

In Experiment 32, 63 minutes after starting heart, stimulation of right vago-sympathetic nerve gave dilation of pupil, but only to one-half maximum size; pupil quickly contracted. Two hours and 36 minutes after starting the heart, atropine failed to dilate the pupil, even after acting a long time.

Experiment 22. Occlusion, 9¾ minutes. Atropine dropped in left eye 2½ hours after release gave normal effect.

The chorda tympani, on stimulation, usually gives a secretion of saliva. The salivary flow returns after release of the arteries, and is often very copious. Atropine may fail to give any result. Stimulation of the vago-sympathetic may cause dilation of the pupil, but usually not to more than one-half of maximum size. The vago-sympathetic in a pup retained its power of producing dilation of pupil two hours and eleven minutes after ligation of the aorta. The flow of lymph from a severed thoracic duct continued throughout the occlusion.

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[23] Krainsky, *Allgemeine Zeitschrift für Psychiatrie*, 1897, liv, 697.

[24] Donath, *Zeitschrift für physiologische Chemie*, 1903, xxxix, 526; *Journal of Physiology*, 1905, xxxiii, 211.

[25] Allen, *Journal of Physiology*, 1904, xxxi, 54.

*The Deportment of the Animal in the Post-Convulsive Period.*—Strictly speaking, there is no post-convulsive period except in those animals which recover completely. For convenience of description, we may speak of the period after cessation of the frequent and violent spasms, as the post-convulsive period. It is characterized by infrequency or total absence of convulsions. There are three phases: (a) complete recovery, (b) partial recovery, and (c) death.

(*a*) *Complete recovery.* Experiment 74. Very large male cat. Ether. Intubation. Occlusion of 8 minutes. Pupils dilate completely in 20 seconds, with lax cornea. Respiration ceases in 70 seconds. First respiratory gasps after release of cerebral arteries in 8 minutes. Pupils contracted in 14 minutes. Natural respiration in 18 minutes. Unusually severe spasms began 29 minutes after release, and lasted 1½ hours. Cat was then able to crawl about, but unable to stand because of paralysis of fore-limbs. Paralysis and wrist-drop entirely gone the next morning (20 hours after release). A peculiar drooping attitude was maintained for 2 days. Afterward the cat appeared entirely normal in every way. When killed by chloroform narcosis 3 months later, lungs showed nodular thickenings and some inflammation. No gross changes in brain.

Complete recovery has resulted after occlusions of five, six, eight, nine and five-sixths, and sixteen and a half minutes.

(*b*) *Partial recovery.* The most interesting case of all comes in this group. Experiment 70. Adult female cat in advanced pregnancy. Ether. Intubation. Occlusion of 10 minutes. Pupils dilated in 15 seconds. Last respiration in 30 seconds. First respiratory gasps 11 minutes after release, spontaneous respiration in 20 minutes. Pupils contracted in 24 minutes. Corneal reflex present in 90 minutes. Convulsions began one hour after release of vessels. Extreme opisthotonus and violent clonic spasms never appeared, but convulsive movements of the limbs were present, without any intermissions, for 3½ days. She lay on her side all the time. Violent spasms resulted whenever the fur was stroked. Walking across the floor caused convulsive starts. The click of the stop watch near her ear, blowing against the fur, or any other slight disturbance caused a convulsive start. The pupils were wide, with light reflex appearing the second day.

On the morning of the fourth day, she was found in a half-sitting posture, head drawn back, and fore-paws outstretched. On the morning of the sixth day, she was able to stand and walk clumsily. Paralysis of limb muscles. but no wrist-drop. She cleaned her paws, purred when spoken to or stroked. Lapped water and milk for the first time since occlusion. Had been fed previously by passing water or milk into her mouth through a tube.

7th day. Walked in a circle, bumping against bars of cage. When placed on floor of room, walked in circle, the size of which was independent of chairs or tables in the way. Bumped into the objects, probably not because of blindness, but because of lack of control of movements. When put in a cleared space, she walked in a circle as before, going two or three times in the same path, as shown by the marks of her fore-feet, which were dipped in water. Little change in deportment on succeeding days.

12th day. Four kittens were born in the morning. One dead when first seen. Others apparently as vigorous as kittens of a normal mother. Old cat paid no attention to them; apparently totally ignorant of their presence if a few inches away, though they mewed loudly. If a kitten came near enough to touch her nose, she licked it with her tongue, fondled it with her paws when nursing, very much as a normal cat would. If a kitten wandered away, she seemed totally unconscious of its existence, and made no effort to bring it back. The kittens died during the next day.

14th day. Light reflex present in some degree. Cat appeared blind, not closing her eyelids until the eyeball was touched. Refused to walk when placed on the floor. Mewed loudly much of the time, and nothing seemed to pacify her. Appeared deaf to ordinary sounds, but total deafness was doubtful.

15th day. Death by inhalation of chloroform because of puerperal infection. Brain and cord showed no gross lesions on post-mortem examination. A considerable quantity of fat present in omentum and mesentery. Stomach contained undigested meat.

Three stages could be distinguished: (1) a period in which all reflexes, with the possible exception of the light reflex, were present; (2) a prolonged period of convulsions, and (3) a period marked by the loss of intelligence, in which the life of reflexes only was present.

Partial recovery, with paralysis of one fore-leg, and death from accidental strangulation on the ninth day, resulted from an occlusion of eight minutes. There were occasional convulsions following unusually violent attempts to move about in the later period, but there was no loss of intelligence. Another occlusion of twenty-two minutes was followed by paralysis of one fore-leg, but not by any loss of intelligence. Death from pneumonia on the seventh day.

A noticeable feature in all these cases of complete or partial recovery was the prominence of the left nictitating membrane. Frequently also the left pupil was more dilated than the right. We have found that the traction on the ligature under the left subclavian artery usually pulls on the ansa subclavia and the left cervical sympathetic. This injury to the left sympathetic nerve trunk probably explains the inequalities in the eyes.

(*c*) *Death.* After long occlusion, death generally follows in thirty hours or less. No post-convulsive period exists in such cases. One peculiar case comes in this group.

Experiment 83. Occlusion 20 minutes. Symptoms of dementia appeared the third day. Pupils wide and staring; choreic movements of the head. Strong tonic or clonic convulsions followed any attempt at quick or unusual movement. Cat mewed loudly when any one came in sight. Would not eat voluntarily; scratched when milk was passed into her mouth through a tube, but swallowed some of it. Sniffed at a piece of meat held in front of her, but ate none of it. The dementia passed away. On fifth day, cat ate normally, responded to a call, and held her head out to be stroked. Convulsions still followed any complicated or rapid movement. Slow movements were fairly well executed. Kept under observation 9 days, but lost sight of during vacation. Future deportment and circumstances of death, which occurred about 10 days later, unknown.

## SUMMARY AND CONCLUSION.

The cerebral circulation was interrupted for periods of three to eighty-one minutes by ligation of the innominate and left subclavian arteries proximal to the origin of the vertebral, in ninety-three cats. Eleven dogs were used in the earlier experiments.

The eye reflexes disappear very quickly and a period of high blood pressure follows the occlusion immediately; vagus inhibition causes cardiac slowing and a fall in blood pressure, followed by a second rise after the vagus center succumbs to anæmia. Respiration stops temporarily (twenty to sixty seconds) after the beginning of occlusion, and then follows a series of strong gasps of the Cheyne-Stokes type, after which it stops until some time after the restoration of the cerebral circulation. The respiratory and vagus centers lose their power of functioning at approximately the same time. Asphyxial slowing of the heart may occur without the agency of the vagus center. The blood pressure slowly falls to a level which is maintained throughout the remainder of the period of occlusion.

The anterior part of the cord and the encephalon lose all function; no reflexes are obtainable. The reflexes of the posterior part of the cord persist; the intravenous injection of strychnine does not affect the anterior part of the cord during the period of occlusion; but does affect the posterior portion of the cord. There is no secretion of tears or saliva, and the intra-ocular pressure is reduced.

The blood pressure falls still more after release of the cerebra arteries, but soon begins to rise. The respiration returns suddenly, two to sixty minutes after restoration of the cerebral circulation, the first gasp being a strong one. The rate gradually increases until rapid enough for natural respiration. The eye reflexes and intra-ocular tension return more gradually, ten minutes to three hours after restoration of the cerebral circulation. The anterior part of the cord recovers its functions gradually. The first reflexes occur only on the same side as the stimulus, crossing of reflexes, to involve the other side, not occurring till later. As a rule, all reflexes return, and a short period of quiet follows. The anterior part of the cord again becomes irritable to strychnine, but succumbs to its action before the normal part. Spasms, of tonic, clonic, or mixed type, then appear, terminating in (a) death, (b) partial or (c) complete recovery. In partial recovery, disturbances of locomotion, such as walking in a circle, paralysis, dementia, loss of sight, hearing, and general intelligence, characterize the post-convulsive period. After complete recovery, there is a return to normal deportment. No gross lesions of the nervous system, other than a congested appearance of the previously anæmic area, were observed.

Transection of the spinal cord stops the spasms below the level of section. Hemisection of the cord stops the spasms on the same side, below the level of section.

Death, without any return of the reflexes after release of the cerebral arteries, has followed an occlusion of seven and one-half minutes. Respiration has returned after an occlusion of one hour. Five animals have recovered completely after an occlusion of seven minutes or more. Only one animal has recovered completely after an occlusion of fifteen minutes. No animal has recovered completely after an occlusion of twenty minutes.

In Herzen's[26] resuscitation of an animal after several hours of cerebral anæmia, there must have been some anastomotic channels to the brain. Mayer's [27] limit of ten to fifteen minutes of cerebral anæmia, beyond which resuscitation is not practicable, is close to the correct one. It appears to us that, in cases of resuscitation two hours after cessation of the heart-beat, (Prus., loc.cit.) the auricles must have kept up a slow but, in some degree, an efficient movement of the blood through the brain. The truth of this suggestion might be tested by introducing some easily recognized, non-diffusible substance into a vein after the heart-beat ceases to affect a manometer, and later searching for it in the brain and other parts of the body. But, whatever the reason, cerebral anæmia in these cases must have been less complete than in our experiments.

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[26] Herzen, *Revue médicale de la Suisse romande*, *1885*, v, 467.

The histological alterations of the cord and brain are now being studied. The results will be published later.

Our thanks are due to Dr. A. Carrel for calling our attention to some of the papers in French.

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[27] Mayer, *Medicinisches Centralblatt*, 1878, xvi, 579.

## TABLE II.

SHOWING LENGTH OF PERIOD OF OCCLUSION, THE TIME OF DISAPPEARANCE, AND THE TIME OF RETURN OF THE REFLEXES AFTER OCCLUSION AND AFTER RESTORATION OF THE CEREBRAL CIRCULATION.

| Number of experiment | Period of occlusion in minutes | Time, in seconds after occlusion of disappearance of reflexes | | | | Respiration first ceases (seconds after occlusion) | Last gasp (minutes after occlusion) | Time, in minutes after restoration of cerebral circulation, of return of reflexes | | | | Minutes after restoration when pupil begins to contract |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Corneal | Lid | Light | Dilation of pupil | | | Corneal | Lid | Light | Respiration | |
| 1 | 5½ | 45 | — | — | 45 | — | — | — | — | — | — | — |
| 2 | 3½ | 30 | — | — | 210 | — | — | 4 | — | — | 4 | 1½ |
| 3 | 4⅝ | 35 | — | — | 35<br>200 | 70 | — | 15½<br>18½ | — | — | 4¾ | 2¾<br>3½ |
| 4 | 7 | 20 | 45 | — | 180 | 165 | — | — | — | — | 2 | — |
| 5 | 6 | 80 | — | 44 | 165 | 120 | — | 18 | 7¾ | 11 | 2⅔ | 7 |
| 6 | 10 | 15 | — | 20 | — | — | 1¾ | — | — | — | 81 | 3½ |
| 7 | 5 | 20 | — | 20 | — | 20 | 2⅝ | 23 | — | 23 | 9½ | 9 |
| 8 | 5½ | 45 | — | 45 | 75 | * | — | — | — | — | — | — |
| 9 | 5½ | — | — | — | — | 27 | — | 27⅔ | — | 22⅔ | 16⅔ | 21 |
| 10 | 12¾ † | 15 | 15 | 15 | — | 20 | — | § | — | — | — | — |
| 11 | 25 | — | — | — | — | — | — | 52 | 55 | 52½ | 38 | 20 |
| 12 | 22 | — | — | — | — | 10 | 7½ | 48 | 48 | 48 | 30½ | — |
| 13 | 6¼ | 10 | — | — | 165 | 10 | 5½ | — | — | — | 31½ | 7¼ |
| 14 | 9⅝ | 27 | 27 | 27 | 240 | * | — | 11⅙ | 11⅙ | 130 | — | — |
| 15 | 5 | 20 | 20 | 20 | 100 | 24 | 2⅝ | 12½ | 12½ | 22⅓ | ½ | 2⅛ |
| 16 | 14 | 75 | 75 | 75 | 120 | 45 | 5¾ | 37 | 32 | 27 | 20 | 11 |
| 17 | 10 | 25 | — | 25 | — | — | 1 5/12 | 25½ | 25½ | 32½ | 5⅙ | 10½ |
| 18 | 20 | 75 | — | — | 75 | — | — | 39 | — | 44 | 5 | 12 |
| 19 | 25½ | 40 | — | — | 120 | 40 | 1⅛ | — | — | 48½ | 27½ | 7½ |
| 20 | 50 | 25 | 25 | 25 | 85 | 25 | 3⅛ | — | — | — | 67½ | 5½ |
| 21 | 81 | 35 | 35 | 35 | 35 | 35 | 11½ | ** | — | — | — | — |
| 22 | 16 | 15 | — | — | 15 | 15 | 2½ | 44½ | — | — | 30 | 5 |
| 23 | 15 | — | — | — | 110 | 18 | — | — | — | — | 8 | 3 |
| 24 | 30 | 15 | — | — | 565 | 10 | 8 | 37¼ | 36¼ | 25¼ | 14¼ | 10¼ |
| 25 | 60′16″ | — | — | — | 14 | 14 | 3⅓ | ** | ** | 44½ | 33⅓ | 25½ |
| 26 | 81 | — | — | — | 30 | 30 | 3¾ | ** | ** | ** | ** | ** |
| 27 | 60 | — | — | — | 120 | 15 | 8 | ** | ** | ** | ** | 28 |
| 28 | 51 | — | — | — | 140 | 30 | 10 | ** | ** | ** | 31 | 41 |
| 29 | 45 | — | — | — | 16 | 44 | 5½ | ** | ** | ** | ** | ** |
| 30 | 45 | — | — | — | 90 | 65 | 9 | ** | ** | ** | 40½ | 7½ |
| 31 | 41 | 25 | — | — | 150 | 40 | 7⅛ | — | — | — | — | 21 |
| 32 | 40 | 25 | — | 25 | — | 15 | 5 | — | — | — | — | 5½ |
| 33 | 31 | 30 | — | — | 270 | 35 | 3 | — | 32 | 18 | 9 | 3½ |
| 34 | 31 | — | — | — | 14 | 35 | 2 | 39 | — | 20 | 18 | 23 right eye<br>86 left eye |
| 35 | 10 | — | — | — | 180 | 120 | 5 | 24½ | — | — | 7 | 13 |
| 36 | 15 | 15 | — | — | 15 | 50 | 3½ | — | — | — | — | 30 |
| 37 | 25¾ | — | — | — | — | — | — | ** | ** | ** | ** | ** |

* Persisted throughout occlusion. † Incomplete occlusion.

§ Reflexes returned before end of occlusion. ** No return of reflexes

TABLE II—*Concluded.*

| Number of experiment | Period of occlusion in minutes | Time, in seconds after occlusion of disappearance of reflexes |  |  |  | Respiration first ceases (seconds after occlusion) | Last gasp (minutes after occlusion) | Time, in minutes after restoration of cerebral circulation, of return of reflexes |  |  |  | Minutes after restoration when pupil begins to contract |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  | Corneal | Lid | Light | Dilation of pupil |  |  | Corneal | Lid | Light | Respiration |  |
| 38 | 61 | — | — | — | 45 | — | — | ** | ** | ** | ** | ** |
| 39 | 8 | — | — | — | — | * | — | 20 | — | — | 23 | 20 |
| 40 | 10′ 7″ | — | — | — | 15 | 30 | — | 89 | — | 48 | 11′38″ | 24 |
| 41 | 8 | — | — | — | 20 | — | — | — | — | — | $7\frac{3}{8}$ | $13\frac{3}{8}$ |
| 42 | 8 | — | — | — | 35 left<br>90 right | 50 | 2′35″ | — | — | — | $8\frac{1}{3}$ | 8 left<br>12 right |
| 43 | 16′35″ | — | — | — | 25 | 50 | 3 | — | — | 72hrs. | $6\frac{5}{12}$ | 10 |
| 44 | $22\frac{1}{8}$ | — | — | — | 90 | — | 5 | ** | ** | ** | $12\frac{1}{2}$ | 42 |
| 45 | $22\frac{1}{4}$ | — | — | — | 240 | — | 5 | — | — | — | 10 | 28 |
| 46 | 11 | — | — | — | 180 | 65 | $5\frac{1}{2}$ | 33 | — | — | $22\frac{1}{2}$ | 33 |
| 47 | $12\frac{1}{2}$† | — | — | — | — | * | — | — | — | — | — | 3 |
| 48 | $11\frac{3}{4}$ | — | — | — | — | — | $7\frac{7}{12}$ | — | — | — | 4 | 4 |
| 49 | $12\frac{1}{4}$ | — | — | — | — | — | — | — | — | — | $18\frac{7}{12}$ | $16\frac{3}{4}$ |
| 50 | $14\frac{1}{8}$†† | — | — | — | — | — | $3\frac{5}{12}$ | ** | ** | ** | ** | ** |
| 51 | 8†† | — | — | — | — | — | $2\frac{1}{8}$ | ** | ** | ** | ** | ** |
| 52 | $6\frac{1}{12}$ | — | — | — | — | — | $1\frac{5}{6}$ | $15\frac{1}{4}$ | — | — | $15\frac{1}{4}$ | — |
| 53 | $6\frac{5}{12}$ | — | — | — | — | — | $2\frac{5}{6}$ | ** | ** | ** | ** | ** |
| 54 | $6\frac{5}{6}$ | — | — | — | 20 | — | 5 | 19 | — | — | $9\frac{1}{2}$ | $9\frac{1}{2}$ |
| 55 | 7 | — | — | — | — | — | $4\frac{2}{3}$ | — | — | — | $2\frac{2}{3}$ | $2\frac{1}{6}$ |
| 56 | 8 | — | — | — | — | 15 | §§ | — | — | — | $9\frac{5}{8}$ | — |
| 57 | $10\frac{1}{6}$ | — | — | — | 55 | — | $1\frac{5}{6}$ | $26\frac{1}{3}$ | — | — | $3\frac{1}{3}$ | 20 |
| 58 | $7\frac{1}{2}$ | — | — | — | 80 | — | $2\frac{1}{8}$ | — | — | — | 4 | 16 |
| 59 | $31\frac{1}{6}$ | — | — | — | — | — | 2 | $47\frac{5}{12}$ | — | — | $15\frac{5}{12}$ | $23\frac{5}{12}$ |
| 60 | $14\frac{5}{8}$ | — | — | — | — | — | $2\frac{5}{6}$ | — | — | — | 26 | 11 |
| 61 | $28\frac{1}{2}$ | — | — | — | 70 | — | — | — | — | — | 39 | $29\frac{1}{4}$ |

The number of the experiment in these tables does not, in general, correspond to the number of the experiment in the series performed.

* Persisted throughout occlusion.

** No return of reflexes.

§§ Secondary series of gasps did not occur.

† Incomplete occlusion.

†† Strychnine injected during occlusion

## TABLE III.

SHOWING PULSE RATE AT INTERVALS DURING OCCLUSION AND AFTER RESTORATION OF THE CEREBRAL CIRCULATION.

| Number of experiment | Minutes from beginning of occlusion | Pulse rate | Minutes after restoration of cerebral circulation | Pulse rate |
|---|---|---|---|---|
| 1 | 16 | 171 | 49 | 150 |
| | 24 | 180 | 99 | 184 |
| | 36 | 171 | 126 | 184 |
| | 50 | 138 | 148 | 162 |
| | — | — | 172 | 204 |
| | — | — | 209 | 180 |
| | — | — | 222 | 160 |
| | — | — | 265 | 171 |
| | — | — | 270 | 168 |
| | — | — | 277 | 167 |
| 2 | 10 | 183 | 31 | 125 |
| | 16 | 184 | 50 | 120 |
| | 27 | 163 | 104 | 120 |
| | 40 | 167 | 122 | 122 |
| | — | — | 162 | 128 |
| | — | — | 186 | 150 |
| | — | — | 202 | 167 |
| | — | — | 246 | 180 |
| | — | — | 271 | 171 |
| 3 | 3 | 108 | 13½ | 140 |
| | 7* | 78 | 25½ | 150 |
| | 9† | 168 | 53½ | 155 |
| | 18½ | 214 | 67½ | 156 |
| | 26½ | 200 | 75½ | 144 |
| | 39½ | 170 | 109½ | 138 |
| | — | — | 112½ | 128 |
| | — | — | 275½ | 150 |
| | — | — | 313½ | 184 |
| | — | — | 350½ | 184 |
| | — | — | 409½ | 166 |
| | — | — | 485½ | 192 |
| | — | — | 515½ | 186 |
| | — | — | 531½ | 200 |
| | — | — | 582½ | 158 |
| | — | — | 626½ | 210 |
| | — | — | 633½ | 209 |
| | — | — | 645½ | 209 |
| | — | — | 683½ | 187 |
| 4 | †† | 155 | 27 | 207 |
| | 25 | 166 | 393 | 168 |
| | — | — | 408 | 172 |
| | — | — | 448 | 170 |
| 5 | 3⅝ | 216 | 16 | 166 |
| | 13 | 203 | 21 | 150 |
| | 20 | 180 | 25 | 162 |
| | 25 | 170 | — | — |
| | 30 | 180 | — | — |
| | 35 | 163 | — | — |
| | 40 | 171 | — | — |
| 6 | †† | 157 | 4 | 120 |
| | 6⅜ | 180 | 7 | 140 |
| | 8 | 180 | 12 | 150 |
| | 18 | 165 | 13½ | 160 |
| | 25 | 96 | 37 | 160 |
| | — | — | 276 | 156 |
| | — | — | 590 | 120 |
| 7 | — | — | ⅓ | 112 |
| | — | — | 6 | 115 |
| 8 | †† | 106 | 1 | 112 |
| | 1½ | 120 | — | — |
| 9 | †† | 130 | — | — |
| | 1½ | 100 | — | — |
| | 2⅛ | 120 | — | — |
| | 4⅝ | 144 | — | — |
| | 10⅝ | 150 | — | — |
| 10 | †† | 156 | ¾ | 156 |
| | 1½ | 120 | 2½ | 150 |
| | 3 | 156 | 4 | 126 |
| | 5† | 126 | 6 | 114 |
| | 6½ | 132 | 7⅝ | 96 |
| | 8½ | 180 | 8½ | 114 |
| | 9 | 162 | 14 | 156 |
| | — | — | 17 | 180 |
| | — | — | 18 | 162 |
| | — | — | 20 | 150 |
| | — | — | 52 | 180 |
| | — | — | 109 | 180 |
| 11 | †† | 156 | 6½ | 150 |
| | 3⅝ | 132 | 12½ | 162 |
| | 6½ | 126 | 35½ | 96 |
| | 8½ | 120 | 37½ | 108 |
| | — | — | 39 | 72 |
| 12 | ¼ | 180 | 2½ | 138 |
| | ½ | 168 | — | — |

* Still gasping.

† Last gasp.

†† Immediately before occlusion.

§ First gasp after restoration of cerebral circulation.

TABLE III—*Concluded.*

| Number of experiment | Minutes from beginning of occlusion | Pulse rate | Minutes after restoration of cerebral circulation | Pulse rate | Number of experiment | Minutes from beginning of occlusion | Pulse rate | Minutes after restoration of cerebral circulation | Pulse rate |
|---|---|---|---|---|---|---|---|---|---|
| 13 | †† | 150 | ¶ | — | 17 | †† | 162 | 1¾ | 156 |
| | ¾ | 156 | 26 | 102 | | ¶ | 186 | 10¾ | 72 |
| | 4¼ | 156 | — | — | | 3 | 186 | 24 | 144 |
| | | | | | | 5 | 168 | — | — |
| 14 | †† | 150 | — | — | 18 | †† | 204 | — | — |
| | ¶ | 150 | — | — | | ¶ | 180 | — | — |
| | 1/10 | 174 | — | — | | 3 | 192 | — | — |
| | ¼ | 162 | — | — | 19 | †† | 210 | 25 | 156 |
| | 2½ | 156 | — | — | | — | — | 90 | 168 |
| | 4 | 162 | — | — | | — | — | 45 hours | 204 |
| 15 | †† | 168 | 20 hours | 138 | | — | — | 7 days | 168 |
| | — | — | 6 days | 162 | | | | | |
| 16 | †† | 100 | — | — | | | | | |
| | 5 | 180 | — | — | | | | | |
| | 9 | 112 | — | — | | | | | |

The number of the experiment in the tables does not, in general, correspond to the actual number of the experiment in the series performed.

†† Immediately before occlusion. ¶ Vagi divided.

# STUDIES UPON CALCAREOUS DEGENERATION.

## II. THE STAINING OF FATTY ACIDS AND SOAPS IN THE TISSUES BY FISCHLER'S METHOD, AND A MODIFICATION OF THE SAME.

## III. CALCIFICATIONS OF THE AORTA IN RABBITS AFTER THE INOCULATION OF ADRENALIN.

## IV. CALCIFICATIONS OF THE MEDIA IN ARTERIES OF THE ELASTIC TISSUE TYPE.

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## II. THE STAINING OF FATTY ACIDS AND SOAPS IN THE TISSUES.

Quite recently Fischer[2] has given us a method, a modification of Benda's copper acetate process, of demonstrating the presence of fatty acids in the tissues, either in the free state or in combination with bases in the form of soaps. By this method the fatty acid compounds are converted into the insoluble copper soaps, which in the presence of hæmatoxylin form a varnish-like substance insoluble in weak acids. The chemistry of the combinations is not as yet fully determined, particularly in regard to the reactions with oleic acid. The process, however, is a very precise one, if the tissue be placed in the copper acetate solution immediately after it is removed from the body, when it is found that the soluble and insoluble soaps, along with the fatty acid in the free state, are picked out very clearly. If, however, as Fischer points out, the tissue be placed in water or an aqueous fixing fluid for some time preceding the treatment with copper acetate, a loss (more or less) of the soluble soaps results.

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[1] This study was aided by a grant from the Rockefeller Institute for Medical Research.

[2] Fischer, *Centralbl.*, *f. Allgem. Pathol.*, 1904, xv, 913.

I say more or less, for, as I have previously pointed out,[3] what constitute soluble soaps in the test tube are not necessarily such in the body.

The method is determinative only for the fatty acid radical, so that the relative quantities of soaps and the free fatty acids cannot be determined. It is true that Fischer advises fixing one portion of the tissue under examination in a formalin solution, while another portion is fixed in a saturated solution of calcium salicylate in 10 per cent. formalin. Both bits of tissue are then treated with the copper acetate solution and the other two fluids in the usual manner. The tissue fixed in the latter solution will demonstrate the combined presence of the free fatty acids and the soaps, while in the former solution some of the soaps have been dissolved out in the fixing fluid, and it was thought that only the free fatty acid radical would remain. However, the calcium soaps, as Langerhans demonstrated them in fat necrosis, and further as have been shown to be present in all pathological processes of calcification, are also almost insoluble formalin in solution, as are also the albuminous compounds of soaps, so that in the formalin-hardened specimen I demonstrated more than the free fatty acid.

The method, however, is very useful in determining the fatty acid radical, free or combined, in microscopic sections, particularly where calcification is progressing in the tissue. The process further has supported my views regarding the process of pathological calcification, that this constantly occurs through the intervention of fatty acids. It is to be noted, as I have pointed out, that the advanced portions of calcified areas no longer contain fatty acids, for the calcium has in these areas formed a more stable compound with carbonic and phosphoric acids.

A modification of Fischer's method of staining the fatty acids brings this still more clearly to light in such sections. If instead of his second solution, which is a saturated solution of hæmatoxylin in absolute alcohol, I substitute a 60 per cent. saturated alcoholic solution of hæmatoxylin, I avoid disturbing the neutral fats present in the degenerated tissues about calcified areas, and thus allow the use of Sudan III or Scharlach R. to stain these.

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[3] Klotz, *American Journal of Physiology*, 1905, xiii, Supp. No. 1, 21.

The process then becomes in short:

1. Fix the tissue and precipitate the fatty acid radical by treating the sections for 1 to 24 hours in the following solution:

Chromalum, 2.5 grams
Formalin, 4%, 100 c.c.
Dissolve by boiling.
While cooling add
Acetic acid, 5 c.c.
and then
Neutral copper acetate (powdered), 5 grams.

2. Thoroughly wash in water.
3. Cut on the freezing microtome.
4. Stain sections in a saturated solution of hæmatoxylin in 60% alcohol for six hours.
5. Wash sections in water and then treat with the following fluid (Weigert's decolorizing fluid) until the tissue becomes a light brown, while the sites of the fatty acid radical remain black.

| | |
|---|---|
| Potassium ferricyanide, | 2.5 grams |
| Borax, | 2.0 " |
| Water, distilled, | 100.0 c. c. |

The sections can then be mounted in Canada balsam.

Blue-black deposits are to be noted in areas where larger quantities of calcium soaps are found.

It is to be remembered that in this method only the copper which is deposited along with the fatty acid stains, and that the structure of the fatty acid compounds as they exist in the tissues is lost. However, this is a minor point, and I am able to determine the smallest particles of the free or combined fatty acid moiety. Now if I wish further to obtain the relation of the fatty acids to the remaining fatty degeneration in the tissue I can stain the sections with Sudan III or Scharlach R.

This combined method of demonstrating the fatty acids and their salts along with the neutral fats is of the greatest service in obtaining a conception of the process of calcification. Thus in the centre of a calcified area the blue-black crystals of the inorganic calcium salts are noted; these are then bordered by an intense black area having outrunners into the surrounding tissue, representing the free fatty acids, the calcium and other soaps; and beyond this area again are to be seen the Sudan stained neutral fats in the degenerating tissues. The tissues which contain fatty acids or their calcium salts are necrotic, and contain no living cells.

This combination of Fischer's fatty acid stain with Sudan III I have used in demonstrating the relation of the fatty acids and their salts to the fatty degeneration in calcified tumors, calcification of the arteries, and phleboliths, and have found all to be of the same character. If in other sections the neutral fats are stained with Sudan III while the calcium salts are blackened with silver nitrate (v. Kossa's method) I can compare the quantity of calcium salts to the fatty acid moiety and roughly determine the amount of calcium soaps present in the tissue. As the process of staining the fatty acids and that of demonstrating the calcium salts both yield black precipitates, I cannot utilize the combined method in the same section. However, as the three staining processes mentioned demonstrate in frozen sections the various stages which calcareous degeneration undergoes, I can follow step by step the stage of fatty degeneration, the production of fatty acids, and the calcium soap formation in all tissues in which pathological calcification is progressing. I have found it advisable to decalcify the tissues in which the fatty acid radical is to be demonstrated, to avoid the confusion with the darkened calcium granules in the finished sections. This is done after applying the copper acetate solution.

## CALCIFICATION OF THE AORTA IN RABBITS AFTER THE INOCULATION OF ADRENALIN.

In a recent number of *Virchow's Archiv*, Scheidemandel[4] discusses the changes occurring in the vessels in rabbits, after the inoculation of adrenalin. Without entering into my own experiments with adrenalin, or touching upon the similarities which these vascular changes bear to arteriosclerosis, it is proper to criticise some of his conclusions which do not conform with the results given by the methods described by me in previous communications.

In his sections of the aorta he finds, when stained with hæmatoxylin and eosin, that between the strands of elastic fibres blue staining granules are deposited in the muscle cells in which the nuclei are still present. In the heavier deposits the whole cells are obliterated and the calcium salts form dark staining bands. The elastic fibres in the areas of calcification, he points out, show a narrowing of their lamellæ, and their staining characters are changed so that they appear granular. In some places he noted tears of the elastic elements. He did not observe any fatty degeneration, nor was he able to determine a calcification of the elastic fibres. He further claims that the elastic fibres are the first to show the degenerative changes in the vessel wall.

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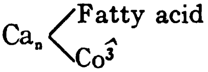
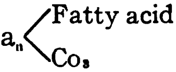
[4] *Virchow's Archiv*, 1905, clxxxi, 363.

In my experience, the deposit of calcium salts within living cells, to the extent that they are stainable with hæmatoxylin, is a very questionable occurrence. The process of calcification is one which does not permit of this, but what I do find, is that in degenerated areas there still exist cells which have not undergone the general necrosis, and in these the nuclei are yet to be seen while the broken down cells about them become calcified. Where living cells exist in calcified areas they do not contain calcium salts to the extent that they will stain with hæmatoxylin. For the detection of finer quantities of calcium, hæmatoxylin stain gives an inadequate reaction, silver nitrate being a more sensitive indicator.

As I have previously pointed out, the process of calcification is preceded by a fatty change, which is followed by a conversion of these neutral fats in the necrotic areas into the fatty acids, and with them the calcium from the blood and lymph forms insoluble soaps.

I have in my possession calcified aortas from rabbits, produced by the inoculation of adrenalin, which present the various stages of the process. The early changes, as has been described by Fischer, are degeneration of muscle fibres. These are shown in my specimens, when stained with Sudan III and hæmatoxlyn, to be fatty degenerations, the fine fat granules lying about the nuclei of the muscle cells. In the more advanced stages the fat granules occupy the greater part of the cells, and the nuclei show degenerative changes. In this condition of the muscle cells the elastic fibres are packed more closely together (Fischer), which leads to a thinning of the vessel wall. If the calcified vessels be stained with Sudan III, I find that the area of calcification is usually the site of a fat-staining deposit—the calcium soap. I say usually, for in the advanced conditions the calcium soaps have been converted into the calcium carbonate and phosphate. However, the simple application of Sudan III to a calcified vessel may show little or no fatty staining material, although such may be present. If in this case I decalcify the vessel before applying the stain I can then demonstrate the fatty substance. I take it that in such cases the calcium forms a combination with both the fatty acids and the carbonate group, thus,  $Ca_n\left<\begin{matrix}\text{Fatty acid}\\ Co_3\end{matrix}\right.$, and that the combination does not stain with Sudan III until I free the fatty acid from the carbonate. When, however, the calcium forms a combination with the fatty acid moiety alone, as I have noted it in several specimens, then I can demonstrate this fatty acid moiety by means of Sudan III. Hence the calcium, deposited in vessels, exists in the combinations, (a) the pure calcium soap, Ca-Fatty acid, (b) the calcium compound with a fatty acid and a carbonate-radical,  $Ca_n\left<\begin{matrix}\text{Fatty acid}\\ Co_3\end{matrix}\right.$, which stains with Sudan III only after decalcifying, and (c) the calcium carbonate, $CaCO_3$, which under no conditions stains with this stain. (Phosphoric acid may replace the carbonate in each of the above compounds.)

That such a fatty acid moiety exists, can also be demonstrated by Fischer's method of staining the fatty acid radical. But similar to the Sudan stain, if the calcium is in combination with both the fatty acid moiety and the carbonate radical, the specimen must primarily be decalcified before applying the reagents. In these specimens the fatty acids and the neutral fats can be demonstrated in the same section by means of the modification of Fischer's stain that I have previously described.

That calcification of the elastic fibres does occur in vessels is not a new subject (Jores, Matusewicz), and I have several examples of the process in human arteries. In the arteriers of rabbits where the elastic fibres are very slender it is impossible to differentiate by means of the hæmatoxylin stain the calcareous granules occurring in the debris of the cells from those which occur in the elastic fibres. It is true in the greater number of aortas showing calcification the elastic fibres are the last to be affected, and they can be distinguished as the wavy refractile lines passing through the dark blue mass. However, using silver nitrate in a weak acetic acid solution, to detect calcium deposits, I find, occasionally, the primary fatty degeneration of the elastic fibres, followed by the deposit of calcium salts. By this method the calcium deposit forms a network of black granules about the muscle bundles. It is the exception to find primary fatty or calcareous degeneration in the elastic fibres, and I must consider the degeneration of the muscle bundles as the rule.

What I would particularly emphasize is that the primary degeneration leading to the subsequent calcification in the aortas of experimental animals, is found in the muscle tissue, and that it is only later that the elastic fibres are changed. A fatty degeneration is to be noted in the muscle fibres long before any macroscopic change is seen in the vessel. The sections of such vessels when stained with Fischer's *fuchselin-scharlach* show that the fatty droplets all lie between the strands of elastic fibres.

Scheidemandel points out that in the calcified aortas of rabbits the elastic lamellæ no longer take the elastic stain characteristically, and that the fibres are granular with light or unstained spaces between the granules. These unstained parts are, as he notes, areas of degeneration, and I would further point out that with the use of *fuchselin-scharlach* these spaces are seen to be the sites of fatty degeneration of the elastic fibres, that is, the degenerations occurring in the elastic fibres are also of a fatty nature, and the changes being similar to those found in the muscle cells these fibres may become the site of calcium deposits.

Of rupture of the elastic fibres, in the vessel wall, I have never been able to convince myself, and must say that those that I have seen, outside of complete tears of one or more tunics in dissecting aneurysms, were artefacts. It is quite evident that, with a degeneration of the muscle fibres, if a rupture of the elastic elements should occur, the intervening space must be filled with blood or coagulated lymph. This is never seen in these specimens. What does happen is that more or less extensive degeneration of the muscle and elastic fibres is found in which these elements no longer take their characteristic stain. It is true that spaces with the ends of the elastic fibres jutting into them from the side are to be seen and that these appear like ruptures of the tissue, but it can be shown with Sudan III and often with the elastic stain that the wavy outlines of the former elastic fibres still stretch through the degenerated area. In human vessels, where degenerations are rarely produced as rapidly as in experimental animals, the new-formed connective tissue fills in the necrotic areas almost as fast as they develop. As Scheidemandel points out, the calcified vessels in rabbits are converted into rigid tubes and no doubt it is in the handling of these fragile sections that the apparent *intra vitam* ruptures in the inner part of the vessel wall result.

In short, the degenerations occurring in the aortas of rabbits inoculated with adrenalin are found chiefly in the muscle cells of the media, and are primarily of a fatty nature, and the inequalities in the staining reaction in the elastic fibres are due also to fatty degeneration of those elements. The calcareous deposits are consequent to the fatty degeneration in that the neutral fats in the degenerated area are converted into fatty acids, which then combine with calcium. The elastic fibres are rarely primarily affected in the degenerative changes, but when they are they may become the primary site of the deposit of calcium. The apparent ruptures of the elastic fibres are either areas of degeneration through which the degenerated fibres still pass, or are artefacts (excluding dissecting aneurysms).

I have to thank Dr. B. Fischer for some of the calcified aortas of rabbits, on which the studies were carried out. A more complete paper dealing with the degenerative processes found in human arteries is to follow shortly.

## CALCIFICATION OF THE MEDIA IN ARTERIES OF THE ELASTIC TISSUE TYPE.

Calcification occurring in the walls of arteries, as one of the degenerative changes in arteriosclerosis, has been extensively described. Particularly well known are the forms of calcification that are found in the intima alone, and which are usually secondary to atheromatous change. Such calcareous deposits have been the subject of discussion ever since arterial disease has been recognized, and later it was shown that these processes remained not alone in the deeper layers of the intima, but advanced into the media.

Jores[5] and Matusewicz[6] have described another form of calcification in arteries, in which isolated patches of the intimal elastic lamina become the site of the deposit. I have met with similar examples, occurring most frequently in the iliac arteries.

Of late Moenkeberg[7] has described a form of calcification that he found in the media of arteries of the muscular type, and noted chiefly in the vessels of the extremities in old people on which clinicians base the diagnosis of arteriosclerosis. His findings are interesting, in that the arterial changes are often confined to these vessels alone, the aorta and its main branches being unaffected. He has found, however, that such degenerative changes do take place in the vessels of the extremities, when more or less arteriosclerosis (intimal disease) is present elsewhere in the body. His statistics show that the femoral artery is most frequently attacked, and that the process having started in one vessel spreads from the primary focus into the branches of the artery. Such a condition as a primary calcification of the media he did not note in any but the vessels of the extremities.

During the routine examination of vessels for degenerative processes in the arterial wall, with the newer technique now at our disposal, I was struck with the frequency with which microscopic calcium deposits were found when the macroscopic examination for the same was entirely negative. I very soon came to the conclusion that the detection of pathological quantities of calcium by the naked eye was not to be relied upon. V. Kossa's method of staining the finer particles of calcium, particularly calcium phosphate, by means of silver nitrate, frequently gave a microscopic picture of calcium salts quite out of keeping with the opinion one formed from the macroscopic examination of the vessel. I have seen specimens in which the aorta was pliable and elastic, with not a trace of calcium salts to the naked eye, while the microscopic section showed the media to be loaded with calcium salts.

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[5] *Ziegler's Beiträge*, 1897, xxi, 211.

[6] *Idem.*, 1902, xxxi, 317.

[7] *Virchow's Archiv*, 1903, clxxi, 141.

Leaving aside for the present the extent and nature of the calcium deposits in the intima, which secondarily invade the media, I wish to discuss the form of calcification found primarily in the media of the aorta and its larger branches, in other words in the vessels of the elastic tissue as distinguished from the muscular type. This form of calcification I have not met with in any other vessels; it differs thus from Moenkeberg's form, which occurs only in arteries of the muscular type.

The age of the patients in which primary calcification of the media of the aorta was found was usually over 65 years; I have encountered three cases under 45 years (aged 39, 41, and 43 respectively). So frequently does a medial deposit of calcium salts occur in old people that it might be ventured that all people over 65 years have it, and that in fully one third of those over 50 years is it present. Sex seems to make no difference.

In the majority of the cases the aorta is the vessel which is attacked, and from here the degenerative process advances into the carotids, innominate, and subclavian arteries. In the latter vessels the condition is as a rule less marked than in the aorta, where the calcareous salts are found uniformly distributed in the media in the different parts of the vessel. No site of predilection was noted in the aorta, but it was found that when one portion of the vessel was affected, calcium salts were found in the media and in other parts also.

It is the common finding that persons over fifty years of age show more or less arteriosclerosis, and hence it would be difficult to draw inferences as to the association of arteriosclerosis at that time of life with the condition here described. In cases, however occurring before fifty years of age, there was a marked absence of gross arterial disease, so that this form of calcification of the media in the vessels of the elastic tissue type stands apart from what is ordinarily understood as arteriosclerosis. Nor is arteriosclerosis to be found in other parts of the body, though I have noted in some, not all the cases, the presence of calcified areas in the media of the vessels of the extremities as described by Moenkeberg.

Hence it would seem that the condition is a form of senile degeneration of the arterial system in which the media of the aorta and its larger branches are involved. This is further borne out by the microscopic examinations.

Macroscopically, as was stated, the vessel may show no change whatever, and the autopsy protocol invariably contains negative reports, except where a concurrent arteriosclerosis affects the other coats of the vessel. The aorta is elastic, not thickened, and the intima is smooth and glistening, while its surface shows a fine sprinkling of fatty granulations so common in advanced life. These slight intimal changes may be considered as the rule in old age, and although they do not represent a physiological process, they play no leading part pathologically. The naked eye inspection of the media and adventitia discloses no change in their structure.

The microscopic examination of frozen sections shows, particularly in the media, interesting changes.

The media is of normal diameter, and at first sight, in hæmatoxylin stained preparations, one is led to believe that no changes have taken place. A closer examination shows in the central zone of the media numerous clusters of fine dark-stained granules, which lie between the elastic fibres. Thick sections, at times, give the impression that the elastic fibres also contain these granules, an impression seen to be erroneous when thinner sections are examined: they lie only in the degenerated muscle fibres. It is to be noted, too, that the muscle tissue in the areas of the dark-stained granulation contains no nuclei, a point which is likely to be overlooked in a careless examination, as the granulation takes the place of the nuclei. In other such areas, which may be looked on as an early stage of the degeneration, the muscle cells are seen to have imperfect outlines, while the nuclei show stages of fragmentation. Amongst those degenerated muscle cells are to be seen, here and there, other cells which have retained their vitality, and hence give the impression that the granular deposit exists in them.

This is not so; more careful study shows that in no case are living cells to be found having within them this deposit of calcium salts. Nor have I noted in any of my specimens a calcification of the elastic fibres such as is found in the calcified media in the vessels of the extremities.

In one case I noted the presence of new formed blood-vessels in the media. While I take it as normal to have blood-vessels in the outer third of the media, yet the presence of numerous capillaries in the media, even though not accompanied by inflammatory infiltration, must be looked on as a reaction to some irritant. The presence of the blood-vessels in the media diminishes the quantity of calcium salts laid down in its vicinity, and the tissue lying about the vessels is entirely devoid of calcareous salts. And, further, the muscle cells lying near the new formed blood-vessel are not degenerated as are those in other parts. It would seem that with the presence of sufficient nutrition the deposit of calcium salts does not take place. This is further borne out by the absence of any deposit in the media bordering the adventitia, and that lying next to the intima. Thus the deposit of calcium is limited to the middle zone of the media.

The inner zone of the media, I take it, is nourished in part at least from the lumen of the vessel, through the intima. Koester[8] has shown that lymphatic spaces pass from the adventitia to and into the intima, and that inflammatory conditions advancing into the vessel wall pass from the adventitia towards the lumen. Confirming this view that some nutriment passes from within outwards into the walls of the arteries, I have noted that in thrombosed vessels, after a week, the intima contains no living cells of its former tissue, and that this tissue and the inner layers of the media are in a state of fatty degeneration. That is if the blood supply within the vessel is cut off, and an effective state of the vasa vasorum persists, the inner layers of the vessel, including the intima and inner strata of the media, become degenerated, and are only later replaced by connective tissue coming in with the newly formed blood-vessels from the adventitia.

Believing, then, that the degeneration and the later deposit of calcium salts in the media rest in part on the nutrition of that coat, it is seen that the greatest deposit of calcium takes place in the middle zone of the media. In the outer one third, where the media is well supplied with capillaries, and in the inner portions of the media supplied through the intima there is a marked absence of the degenerative process.

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[8] *Berlin klin. Wochensch.*, 1876, p. 454.

Moenkeberg found in his cases of calcification of the arteries of the extremities, that the muscle fibres first showed a fatty degeneration. A similar fatty degeneration is also to be seen in the aortas of the cases under description. The fat granules are first found lying about the nuclei of the muscle fibres, while after the degeneration has advanced, and the cell has become filled with the small fat droplets, the nucleus becomes lost. The cell itself is later broken up, leaving the fat granules lying in the interstices of the remaining muscle cells. Thus it is often seen that the intact muscle fibres are surrounded by aggregations of these fine fat particles, which have been described as arising out of the lymph surrounding the cells. With the loss of more or less of the muscle tissue, by this process of fatty degeneration, I find a definite shrinkage at the site where they previously existed; on account of the blood pressure within the vessel, the laminæ are pressed closer together, and the remains of the fatty degenerated muscle fibres are found as clusters of fat droplets in the clefts between the remaining muscle cells. It is further to be noted that these aggregations of fat granules of former muscle cells are found lying next to the elastic fibres of the media, the elastic fibres themselves being unaffected. In none of the specimens that I have noted, in which a calcification of the media of the vessels of the elastic tissue type alone was present, have I seen fatty degeneration of the elastic fibres in the media. Thus it is seen that the disease affects primarily the muscle fibres.

With the modification of Fischer's method of detecting fatty acids and soaps (page 324) I was able, after decalcification of the specimens, to demonstrate that these same areas in the media are areas of advanced fatty degeneration. They give the reactions both for fatty acids and soaps.

That the deposit which I have demonstrated, by the use of silver nitrate, is calcium, is shown by its staining a dark blue with hæmatoxylin, its solution in hydrochloric acid, and the formation of crystals with sulphuric acid.

The best microscopic specimens demonstrating the calcium granules are obtained by the silver nitrate method, in which the granules are seen to be arranged in clusters parallel to and often closely bordering upon the elastic fibres. The elastic fibres are always to be traced as uninterrupted strands through these areas of degeneration. That the elastic fibres resist degenerative processes to a greater extent than the muscle fibres, I have noted also in other arterial diseases. Thus, too, in the calcified areas of the arteries of the extremities the muscle fibres are the first to be affected, and in the early stage the calcification of these fibres is similar to the process in the muscle fibres in the aorta. The muscle fibres undergo an early fatty degeneration, which is later followed by a deposit of lime salts. As the calcification of the muscle cells advances the granules of lime salts become confluent and form larger masses. In the calcified arteries of the extremities, described by Moenkeberg, it is seen that the outer border of the calcareous deposit has a similar appearance to the calcification in the arteries of the elastic tissue type, the difference between them being that in the former the process goes on much further, attacking the elastic fibres and later forming macroscopic deposits of calcium. Macroscopic deposits of calcium in the media alone of the aorta I have not so far encountered and am led to doubt if they ever occur.

The process of calcification in itself is similar to that found in other degenerative processes. One has to do essentially with a fatty degeneration of the specific cells, followed by death of the cell and a fatty acid stage, in which calcium soaps are laid down. A later conversion of the calcium soaps into the calcium phosphate and carbonate then results.

The intima, as I have said, showed no macroscopic change except in such cases as exhibited a concurrent intimal arteriosclerosis, and in these latter cases the medial disease was equally pronounced in the portions of the aorta lying beyond the intimal plaques. However, in all cases showing calcareous deposit in the media, the intima was found to be fatty, the degeneration being most marked in the deep, or musculo-elastic layer. In the early cases the changes in the intima are mostly a hypertrophy of the musculo-elastic layer, with more or less of a fatty degeneration attending it. The connective tissue layer of the intima is not thickened, neither does it show any degenerative changes. Similarly no constant characteristics were noted regarding the elastic fibres in the intima, which at times showed an increase in the lamellæ of the internal elastic lamina, while at other times this lamina showed fatty changes. The changes in the elastic fibres in the intima were related more or less to the character and condition of the elastic fibres in the media, being increased when the latter were in a state of granular degeneration.

In conclusion I would say that the calcification of the media of vessels of the elastic tissue type resembles that found in the arteries of the extremities (the vessels of the muscular type) in that the process of calcification is confined to the media, but differs from it in that the deposit of calcium salts does not go beyond the granular stage, and the muscle fibres alone are involved in the process of degeneration, the elastic fibres remaining unaffected. Macroscopically there is no change in the media, while microscopic sections show the media to be loaded with a granular calcium deposit. This process, which stands in close relation with the nutrition of the vessel wall, is a common one in persons over fifty years of both sexes, and almost constant in the aortas of those over sixty-five years of age.

# THE INFLUENCE OF TEMPERATURE UPON THE RATE OF REACTION (HÆMOLYSIS, AGGLUTINATION, PRECIPITATION).[1]

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It becomes more and more evident each year, as we advance in the field of immunity, that the bodies which are concerned follow in their action the same laws as other chemical bodies, and therefore must be examined by the methods of modern chemistry. One important step toward the simplifying of this study was made by Ehrlich, when he defined substances of which the action could be measured by test tube experiments. This method allows us to vary the experimental conditions to such an extent that we have control over a whole series of different factors (temperature, dilution, medium, etc.). It is a common observation of all those workers who have investigated hæmolysis, agglutination, precipitation, etc., that these are not simple phenomena and the important bearing which such studies have upon the whole theory of immunity makes it necessary that careful investigation be carried out upon the factors which influence them. Among the factors which play a rôle in chemical reactions, few equal temperature in importance. In the study of hæmolysins and agglutinins it was very early recognized that their activity to a great extent depended upon temperature, increasing as the temperature increased, but diminishing or disappearing at low temperatures.

On account of the great theoretical and practical interest which the study of the dependence of the rate of reaction upon the temperature presents, we have carried out a series of experiments with different substances. The experiments include a certain number of hæmolysins (alkalis, acids, bacteriolysins, snake venoms) and some agglutinins and precipitins of different kinds. The ordinary physico-chemical method used for this purpose would be as follows: To determine the rate of reaction, for example, between a hæmolysin and the erythrocytes at different temperatures, and then find the relation between these rates of reaction and the corresponding temperatures. However, this method was not applicable to this investigation, since as yet we do not know the nature of the process which takes place when the hæmolysins and erythrocytes are brought together, and therefore lack the formulas which express these phenomena. For this reason it became necessary to employ another method. The procedure will perhaps be more easily understood by the detailed description of one experiment.

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[1] See Toxines et Antitoxines : L'influence de la température sur la vitesse de réaction. I, par Madsen et Walbum; II, par Madsen et Noguchi. *Académie Royale des Sciences et des Lettres de Danemark, Séance du 20 Mai, 1904.*

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The object of the experiment was to determine the hæmolytic action of sodium hydrate at different temperatures. Seven water-baths with constant temperatures of 39.3°, 34.3°, 30.2°, 27°, 21.2°, 16°, 13.7° C. were prepared. Seven stands, each with six test tubes containing eight cubic centimeters of a one per cent. emulsion of erythrocytes of the horse were put in the baths. When the erythrocytes had been allowed to subside in order to avoid too quick mixture, and the fluid had obtained the surrounding temperature, the amounts of 0.2*n* sodium hydrate indicated in Table I were added as fast as possible (by three experimenters working simultaneously); the tubes were then shaken. Ten minutes afterwards all the stands were taken up at once. It was found that the degree of hæmolysis in the third tube at 39.3° C. (containing 0.06 c.c. of 0.2*n* sodium hydrate) corresponded to a stage intermediate between the second and third tube at 34.3° C. (0.1 c.c.–0.08 c.c.), but nearer the third, and it was estimated that hæmolysis in the third tube at 39.3° would correspond to that caused by .085 c.c. at 34.3° C. In a similar way it was estimated to correspond at 30.2° to 0.11 c.c., at 27° to 0.12 c.c., at 21.2° to 0.22 c.c., at 16° to 0.4 c.c., and at 13.7° to 0.55 c.c.

These results are collected in Table II under the heading 10*m*. In the first column are given the temperatures, in the second

($\frac{1}{c}$ obs.) the observed values, in cubic centimeters, producing the same degrees of hæmolysis. It is obvious that a much smaller amount of sodium hydrate is required at high than at a low temperature to produce the same effect. In the case of a great number of chemical substances, Arrhenius found that the increase of the rate of reaction with increasing temperature could be expressed by the following formula:

$$\frac{c_1}{c_2} = e^{\frac{\mu}{R}\left(\frac{T_1 - T_2}{T_1 T_2}\right)}$$

In the formula $c_1$ and $c_2$ indicate the concentrations (rates of reaction) at the temperatures $T_2$ and $T_1$; $\mu$ is a constant, $T_1$ and $T_2$ the absolute temperatures, and $R$, expressed in calories, is 2. The experiment which has been described, like most of our experiments with hæmolysins and agglutinins, can be expressed by this formula. The values calculated in this way are given in the tables under $\frac{1}{c}$ calc. Below is indicated the constant $\mu$ in calories. As a rule the observed values correspond with the calculated within the error of experiment.

The difference in the hæmolytic action at low and high temperature expressed by the constant $\mu$ is most marked immediately after the addition of the blood emulsion and decreases with increasing time. The final effect—the highest which can be obtained with the hæmolytic substance under the given conditions (medium concentration of blood corpuscles)—is the same for all temperatures; but at low temperatures a much longer time is of course required. The constant $\mu$ decreases with increasing time, following apparently a hyperbolic curve, and is zero when the final hæmolytic effect is obtained. Theoretically it would be most correctly determined for the time, zero. This being impossible it must be extrapolated from the other values.

The two *strong alkalis*, sodium and potassium hydrate, of which the degree of dissociation is about the same, act in almost the same manner (Tables II and III). On the contrary there is a marked difference in the case of ammonium hydrate (Table IV). Corresponding to the much smaller rate of reaction of this body, the value of $\mu$ is in the beginning much larger, being about double that for sodium and potassium hydrate.

The *final effect* of all alkalis is the same as is obvious from Tables V, VI, and VII. These tables present the hæmolysis produced by *equivalent* amounts of different bases and acids. The erythrocytes were suspended in physiological salt solution or in isotonic cane sugar solution. The numbers 15, 30, 60, etc., indicate the time in minutes required for the production of total hæmolysis. The rate of reaction is the same with equivalent amounts of sodium, potassium, lithium, and barium hydrates, but is much smaller with ammonium hydrate. But if the reaction is increased by shaking the tubes and keeping them some time at 37° C., the difference is diminished and the final limit between the minimal effect and no effect will be about the same. In the tables this limit is marked by a horizontal line. It is obtained after the tubes have been (1) observed six hours at the temperatures indicated in the table; (2) shaken; (3) kept twenty hours at 35.5° C.

The investigation of the hæmolysis produced by *acids* is rendered rather difficult by the strong agglutination and the considerable alteration of the hæmoglobin to which they give rise. Therefore it is difficult to get regular values, but the data given are sufficient to demonstrate the very great differences in the rates of reaction of different acids. Their final effect is nearly the same, but it is often much obscured by agglutination which causes precipitation of the erythrocytes before they are dissolved. This is perhaps one of the reasons why the limit for the "weak" acids is with a little greater amounts than with the "strong" acids. Constant shaking of the tubes with acetic acid will put the limit for this acid at a level with that of sulphuric or hydrochloric acid.

It is interesting to note that both for acids and for alkalis the final limit is obtained with about equivalent quantities, generally a little smaller in the case of alkalis. It is evident that the action of these substances is not directly dependent on the ions. The phenomenon recalls that of the formation of a salt. Acids with a very low degree of dissociation (boric acid) have no hæmolytic action.

Measuring the hæmolytic power of the *acids*, for the above mentioned reasons, is much more difficult than measuring that of alkalis. If to a series of test tubes, containing equal quantities of a one per cent. emulsion of erythrocytes of the horse, varying amounts of an acid (for example, sulphuric acid) be added, the following is observed: The higher doses will produce a strong brown discoloration of the erythrocytes which clump together and subside, having lost part of their color. With smaller doses total hæmolysis occurs and the color remains almost unchanged. Still further down in the series a decreasing hæmolysis and partial agglutination is observed. Smaller doses produce agglutination alone without hæmolysis.

The rate of action of the readily dissociated acids is so fast that it can hardly be measured. The organic acids generally present less difficulty. We have examined the four lowest members of the fatty acids, namely, *formic*, *acetic*, *propionic*, and *butyric acid*. From Tables VIII, IX, X, and XI, it is obvious that with these acids also the observed values correspond to the calculated. There is a marked difference between formic acid and the three other acids, the constant $\mu$ for the former being much smaller and corresponding to the greater rate of reaction. With similar results we have examined *maleinic*, *citraconic*, and *itaconic acid*. In this instance also the acid with the lowest degree of dissociation, the itaconic acid, presents the highest constant, but here too no direct proportion between the rate of reaction and the constant of dissociation is found (Tables XII, XIII, and XIV). Furthermore our investigations include some other hæmolytic bodies: *oleinic acid*, *sodium-oleate*, and *triolein*. Tables XV, XVI, and XVII show that these bodies follow the general rule.

In the above experiments the influence of the temperature has been investigated at different stages of the reaction (ten, twenty, thirty, etc., minutes after its beginning). Some experiments have been carried out, in which only a single stage, suitable for the measurement, has been taken. In this way have been examined: The dissolving effect of *streptolysin* upon the red blood corpuscles of the horse (Table XVIII); the agglutinating action of *ricin* upon rabbit blood (Table XIX); the precipitation produced by strong *sulphuric acid* in a solution of albumen

(Table XX); the agglutination of horse blood by ***sublimate*** (Table XXI); and the precipitating action of a ***specific egg-precipitin*** upon egg-albumen (Table XXII). The agglutinating action of ***coli-agglutinin*** is highly accelerated by increasing the temperature, as the high initial constant of Table XXIII shows. But the longer the time that elapses after the beginning of the reaction, the less the difference observed and the final effect of a given amount of agglutinin is the same at all temperatures. Table XXIV gives an example of a phenomenon which is rather often observed in experiments of this kind: The constants of the measurements, made ten, twenty, and thirty minutes after the beginning of the reaction, decreased regularly; but they subsequently increased very much in the determinations made after forty and fifty minutes. The first group of experiments were carried out in the forenoon, the last mentioned some hours later. Perhaps during this interval a considerable change had occurred in the emulsion of coli-bacilli, of such nature that the influence of the temperature had become much more important. The ***agglutinin of Bacillus typhosus*** seems in this respect to be quite analogous to the coli-agglutinin (Table XXV).

Among the bacterio-hæmolysins the ***vibrio-lysin*** (Table XXVI) seems to follow the general rule.[3] Our first experiments with ***tetanolysin*** seemed to indicate that this body follows the same rule within the temperatures 35.3° C. and 10.8° C. (Table XXVII). This tetanolysin was the same which had served for all the previous experiments in this Institute. In former experiments irregularities of action had never been observed, except when the lysin had become very weak. Now the greatest effect was not produced in the tubes containing the highest quantity of lysin, but in those with the lysin in much weaker concentration. Some examples of this behavior will be found in the following experiments: To twenty-seven tubes, with one per cent. horse blood amounts of lysin decreasing from 1 c.c. to 0.0025 c.c. are added (Table XXVIII). After ten minutes there is almost complete hæmolysis with 0.1 c.c.; from this tube the hæmolysis decreases in the series both upwards and downwards; almost the same degree of hæmolysis is observed with 1 c.c. and with 0.07 c.c. Three and a half hours later the series presents the following result: Total hæmolysis with 0.1 c.c. and 0.07 c.c. The tubes with smaller quantities show a gradually decreasing hæmolysis. In the tubes with higher amounts of lysin the hæmolysis is strongest with 0.13 c.c. gradually decreasing to a minimum at 0.3 c.c. and 0.25 c.c.; from this point upwards the hæmolysis again increases, and with 1 c.c. almost total hæmolysis occurs. After a time all the tubes containing over 0.1 c.c. presented total hæmolysis, and therefore we would not have had any idea of these great irregularities in the rate of reaction if we had made our examination only on the following day.

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[3] This vibrio-lysin is made with a vibrio, for which we are indebted to the kindness of Dr. Kraus in Vienna; the same is true for the staphylococcus, mentioned below.

These irregularities are not due to the weakness of this lysin, as we have found similar irregularities with quite fresh cultures. Thus Table XXIX shows an experiment in which hæmolysis at 90° C. and 16° C. advances regularly; $H_{10}$, $H_{20}$, $H_{40}$ indicate the time in minutes required for total hæmolysis. But at 26 C.° is found a broad zone between 0.25 c.c. and 0.05 c.c in which total hæmolysis occurs after ten minutes, and upward and downward in the series decreasing lysis is found. At 40° C. after ten minutes total hæmolysis occurred in the tubes with 0.8, 0.5, 0.3, 0.2, 0.08 c.c. and in all the tubes from 0.04 to 0.015 c.c. In the intermediate tubes the hæmolysis was very much less.

As all the experiments with this lysin presented similar results, it was extremely difficult to compare the effect at different temperatures. There seems to exist a maximum around 31.7° C. while the action is much smaller at other temperatures. Some examples are given in Tables XXX and XXXI. In several series two values are given indicating the presence of the above mentioned irregularities.

It might be thought that the decrease of the hæmolytic action at higher temperatures, 36.5° and 41.7° C., might possibly be due to a weakening of the tetanolysin at these temperatures. However some experiments, carried out specially to settle this question, showed another curious phenomenon: in a certain number of cases the activity of the lysin was increased considerably by a short heating to 37° C. As an example the following experiment is given. The broth medium usually employed (0.5 per cent. glucose, 2 per cent. pepton, 1 per cent. sodium chloride) was inoculated February 2 with tetanus bacilli and kept under paraffin at 35.5° C. until February 21. It was then taken from the incubator and kept at low temperature, about 4° C. Three days afterwards it was heated to 37° C. and the hæmolytic effect then tested. In Table XXXII $\frac{1}{c}$ indicates those amounts of the culture, heated to 37° C., during the time $t$, which have the same action. The experiments have been carried out during a series of successive days and have shown that the increase of the hæmolytic power is sometimes rather considerable. Other tetanus cultures present analogous phenomena. The experiment described by Table XXXIII shows how heating to 37° C. during ten minutes can quadruple the hæmolytic power of the culture.

We have, however, obtained several times tetanus cultures without these irregularities and the influence of the temperature has conformed to the usual rule.

Another lysin, *staphylolysin*, showed the peculiar phenomenon described above, the highest hæmolytic effect not occurring at the highest temperature. A given quantity produced the maximal action between 20° C. and 30° C., the effect being considerably smaller at higher or lower temperatures (Table XXXIV).

With another body, which has played an important rôle in the hæmolytic experiments of recent years, *lecithin*, hæmolytic action does not seem to depend very much on temperature. Table XXXV presents observations after 20, 30, 50, 60, 90, 140, and 330 minutes at four different temperatures, namely, 0.1°, 11.1°, 24.05°, and 36.7° C. The figures in columns indicate hæmolysis in percentages.[4] The process proceeds rather regularly; the effect seems to be about the same at 0.1° C. and 36.7° C. and a little less at the intermediate temperatures.

In the case of *cobra venom* there is a little increase of hæmolysis at higher temperature; a minimum seems to exist between 14° C. and 24° C. (Table XXXVI). On the contrary hæmolysis by the venom of *Ancistrodon piscivorus* (water moccasin) is considerably greater at low than at high temperature (Table XXXVII). It is interesting to remember that this venom is used by the water moccasin to poison chiefly cold-blooded animals.

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[4] Arrhenius and Madsen: Physical Chemistry Applied to Toxins and Antitoxins, Festskrift ved Indvielsen af Statens Seruminstitut, Copenhagen, 1902.

The experiments which have been communicated must be considered only as preliminary steps into a field which has not yet been investigated; owing to the great errors of experiment and to our still very incomplete methods of measurement the data cannot be definitive. A striking feature is, that among a number of experiments which have corresponding results are found irregular series, which do not follow the usual course. In such instances it seems reasonable to think that influences of catalytic nature have had a part, increasing or decreasing the rate of reaction.

Our experiments show that hæmolysis, agglutination, and precipitation by a great many different substances increase with increasing temperature, following the same formula, which applies to other chemical phenomena:

$$\frac{c_1}{c_2} = e^{\frac{\mu}{R}\left(\frac{T_1 - T_2}{T_1 T_2}\right)}$$

This is true for the hæmolysis by the hydrates of sodium, potassium, and ammonia, by formic, acetic, propionic, butyric, maleinic, citraconic, itaconic, and oleinic acids, by sodium oleate and by triolein, and furthermore by streptolysin and vibrio-lysin. It is true for the agglutination of the erythrocytes by ricin and mercuric chloride, for the precipitation of albumen by sulphuric acid and by specific precipitin; and also for the bacterial agglutination by the agglutinins of Bacillus coli and Bacillus typhosus. Still the increase of the action with the temperature can only reach a certain limit, partly owing to the destruction of the medium (erythrocytes, bacteria), partly owing to the weakening of the active body itself (bacteriolysin, agglutinin).

On the contrary, the tetanolysin showed peculiarities, for example, a maximum activity at 32° C.; staphylolysin showed a maximum activity between 20° C. and 30° C.; with lecithin and cobra venom hæmolysis at low and high temperature seems to proceed with about equal rapidity and to be more rapid than at the intermediate temperatures; the venom of the water moccasin acts most strongly at low temperatures.

TABLE I.

ACTION OF 0.2*n* NaOH UPON 1 PER CENT. EMULSION OF HORSE BLOOD AT DIFFERENT TEMPERATURES.

| | 39·3° | 34·3° | 30.2° | 27.° | 21.2° | 16° | 13·7° |
|---|---|---|---|---|---|---|---|
| 1 | 0.1 c.c. | 0.13 c.c. | 0.2 c.c. | 0.2 c.c. | 0.3 c.c. | 0.8 c.c. | 0.8 c.c. |
| 2 | 0.08 " | 0.1 " | 0.17 " | 0.17 " | 0.25 " | 0.6 " | 0.6 " |
| 3 | 0.06 "× | 0.08 "×§ | 0.13 " | 0.13 "× | *0.2 "×§ | 0.5 " | 0.5 " |
| 4 | 0.05 " | 0.06 " | 0.1 "×§ | 0.1 " | 0.17 " | 0.4 " | 0.4 " |
| 5 | 0.04 " | 0.05 " | 0.08 " | 0.08 " | 0.13 " | 0.3 " | 0.3 " |
| 6 | 0.03 " | 0.04 " | 0.06 " | 0.06 " | 0.1 " | 0.25 " | 0.25 " |

* Corresponds to an amount between two that are given but nearer the larger.

× Corresponds to an amount midway between two that are given.

§ Corresponds to an amount between two that are given but nearer the smaller.

TABLE II.

0.2 *n* NaOH.

| **10*m*** | | | **20*m*** | | | **30*m*** | | |
|---|---|---|---|---|---|---|---|---|
| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. | Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. | Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
| 39·3 | 0.06 | 0.057 | 39·3 | 0.055 | 0.054 | | | |
| 34·3 | 0.085 | 0.084 | 34·3 | 0.07 | 0.069 | 34·3 | 0.06 | 0.057 |
| 30.2 | 0.11 | 0.12 | 30.2 | 0.085 | 0.086 | 30.2 | 0.07 | 0.071 |
| 27.0 | 0.12 | 0.155 | 27.0 | 0.1 | 0.1 | 27.0 | 0.065 | 0.083 |
| 11.2 | 0.22 | 0.26 | 21.2 | 0.13 | 0.14 | 21.2 | 0.115 | 0.116 |
| 26.0 | 0.4 | 0.4 | 16.0 | 0.185 | 0.184 | 16.0 | 0.15 | 0.15 |
| 13·7 | 0.55 | 0.5 | 13·7 | 0.26 | 0.21 | 13·7 | 0.19 | 0.17 |
| $\mu = 15190$ | | | $\mu = 9486$ | | | $\mu = 9441$ | | |

| **40*m*** | | | **50*m*** | | | **60*m*** | | |
|---|---|---|---|---|---|---|---|---|
| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. | Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. | Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
| 39·3 | 0.04 | 0.04 | 38.5 | 0.043 | 0.042 | | | |
| 34·3 | 0.05 | 0.05 | 34·3 | 0.048 | 0.049 | 34·3 | 0.045 | 0.045 |
| 30.2 | 0.1 | 0.063 | 30.8 | 0.07 | 0.057 | 30.8 | 0.055 | 0.052 |
| 27. | 0.07 | 0.073 | 26.8 | 0.065 | 0.067 | 26.8 | 0.065 | 0.061 |
| 21.2 | 0.1 | 0.1 | 21.1 | 0.07 | 0.085 | 21.1 | 0.07 | 0.077 |
| 16.0 | 0.13 | 0.13 | 15.8 | 0.1 | 0.107 | 15.8 | 0.09 | 0.096 |
| 13·7 | 0.17 | 0.15 | 14.6 | 0.115 | 0.112 | 14.6 | 0.1 | 0.12 |
| $\mu = 9210$ | | | $\mu = 7415$ | | | $\mu = 7185$ | | |

| *80m* | | | *100m* | | | *180m* | | |
|---|---|---|---|---|---|---|---|---|
| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. | Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. | Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
| 38.5 | 0.035 | 0.038 | 38.5 | 0.04 | 0.045 | 38.5 | 0.04 | 0.04 |
| 34.3 | 0.045 | 0.044 | 34.3 | 0.05 | 0.05 | 34.3 | 0.04 | 0.045 |
| 30.8 | 0.055 | 0.05 | 30.8 | 0.06 | 0.058 | 30.8 | 0.057 | 0.05 |
| 26.8 | 0.055 | 0.059 | 26.8 | 0.07 | 0.067 | 26.8 | 0.06 | 0.057 |
| 21.1 | 0.085 | 0.074 | 21.1 | 0.085 | 0.081 | 21.1 | 0.07 | 0.068 |
| 15.8 | 0.07 | 0.091 | 15.8 | 0.085 | 0.098 | 15.8 | 0.07 | 0.081 |
| 14.6 | 0.1 | 0.095 | 14.6 | 0.1 | 0.1 | 14.6 | 0.085 | 0.085 |
| $\mu$ = 6907 | | | $\mu$ = 6263 | | | $\mu$ = 5663 | | |

## TABLE III.

0.2*n* KOH.

| *10m* | | | *20m* | | | *30m* | | |
|---|---|---|---|---|---|---|---|---|
| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. | Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. | Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
| 38.8 | 0.11 | 0.1 | 38.1 | 0.065 | 0.076 | 38.1 | 0.1 | 0.096 |
| 35.7 | 0.14 | 0.12 | 35.9 | 0.085 | 0.084 | 35.9 | 0.13 | 0.11 |
| 28.8 | 0.17 | 0.18 | 28.5 | 0.17 | 0.12 | | | |
| 25.4 | 0.21 | 0.23 | 25.4 | 0.15 | 0.14 | 25.4 | 0.17 | 0.17 |
| 19.5 | 0.33 | 0.34 | 19.4 | 0.2 | 0.195 | | | |
| 15.3 | 0.45 | 0.45 | 15.2 | 0.225 | 0.245 | 15.2 | 0.25 | 0.28 |
| 13.4 | 0.5 | 0.52 | 14.1 | 0.275 | 0.26 | 14.1 | 0.3 | 0.29 |
| $\mu$ = 11710 | | | $\mu$ = 9210 | | | $\mu$ = 8288 | | |

| *40m* | | | *60m* | | | *120m* | | |
|---|---|---|---|---|---|---|---|---|
| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. | Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. | Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
| 38.6 | 0.06 | 0.06 | 38.1 | 0.07 | 0.076 | | | |
| | | | 35.9 | 0.085 | 0.081 | 35.6 | 0.05 | 0.053 |
| 28.6 | 0.085 | 0.09 | 28.5 | 0.1 | 0.1 | 28.4 | 0.065 | 0.064 |
| 25.4 | 0.12 | 0.11 | 25.4 | 0.13 | 0.12 | 25.3 | 0.07 | 0.07 |
| 19.4 | 0.13 | 0.14 | 19.4 | 0.1 | 0.14 | 19.4 | 0.09 | 0.084 |
| 15.2 | 0.17 | 0.17 | 15.2 | 0.17 | 0.17 | 15.1 | 0.09 | 0.095 |
| 13.7 | 0.18 | 0.18 | 14.1 | 0.17 | 0.173 | 14.5 | 0.1 | 0.097 |
| $\mu$ = 7968 | | | $\mu$ = 6078 | | | $\mu$ = 5164 | | |

**180*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 39.8 | 0.04 | 0.04 |
| 35.6 | 0.037 | 0.044 |
| 29.3 | 0.05 | 0.051 |
| 25.6 | 0.06 | 0.055 |
| 20.3 | 0.065 | 0.062 |
| 15.3 | 0.07 | 0.07 |
| 13.8 | 0.07 | 0.073 |

$\mu = 4145$

## TABLE IV.

0.5*n* $NH_4OH$.

**10*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 39.5 | 0.04 | 0.043 |
| 34.8 | 0.085 | 0.083 |
| 29.7 | 0.17 | 0.17 |
| 25.9 | 0.3 | 0.3 |
| 21.0 | 0.60 | 0.64 |

$\mu = 26760$

**20*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 39.2 | 0.03 | 0.029 |
| 34.8 | 0.05 | 0.05 |
| 30.2 | 0.085 | 0.081 |
| 25.7 | 0.19 | 0.17 |
| 21.0 | 0.3 | 0.32 |
| 15.7 | 0.55 | 0.69 |

$\mu = 24360$

**30*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ cak |
|---|---|---|
| 39.5 | 0.0275 | 0.0225 |
| 34.8 | 0.035 | 0.039 |
| 29.7 | 0.07 | 0.072 |
| 25.9 | 0.13 | 0.12 |
| 21.0 | 0.25 | 0.225 |
| 15.2 | 0.5 | 0.5 |
| 11.0 | 0.7 | 0.89 |

$\mu = 23020$

**40*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 39.1 | 0.02 | 0.019 |
| 34.6 | 0.03 | 0.031 |
| 30.7 | 0.05 | 0.048 |
| 25.9 | 0.085 | 0.086 |
| 21.3 | 0.17 | 0.15 |
| 16.1 | 0.3 | 0.3 |
| 12.2 | 0.45 | 0.49 |

$\mu = 21640$

**60*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 39.2 | 0.019 | 0.014 |
| 34.8 | 0.024 | 0.022 |
| 30.2 | 0.035 | 0.035 |
| 25.7 | 0.05 | 0.056 |
| 21.1 | | |
| 15.7 | 0.17 | 0.17 |
| 12.2 | 0.26 | 0.26 |

$\mu = 19150$

**80*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 39.2 | 0.025 | 0.02 |
| 34.8 | 0.03 | 0.03 |
| 30.2 | 0.04 | 0.046 |
| 25.7 | 0.07 | 0.069 |
| 21.1 | 0.1 | 0.11 |
| 15.7 | 0.18 | 0.18 |
| 12.2 | 0.3 | 0.26 |

$\mu = 16660$

**100*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 39.1 | 0.02 | 0.018 |
| 34.6 | 0.025 | 0.025 |
| 30.7 | 0.025 | 0.034 |
| 25.9 | 0.045 | 0.051 |
| 21.3 | 0.06 | 0.075 |
| 16.1 | 0.115 | 0.12 |
| 12.2 | 0.17 | 0.17 |

$\mu = 14920$

**120*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 39.1 | 0.017 | 0.017 |
| 34.6 | 0.0225 | 0.0225 |
| 30.7 | 0.0275 | 0.029 |
| 25.9 | 0.04 | 0.04 |
| 21.3 | 0.06 | 0.055 |
| 16.1 | 0.1 | 0.079 |
| 12.2 | 0.17 | 0.1 |

$\mu = 13720$

**180*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 39.0 | 0.015 | 0.015 |
| 34.7 | 0.0185 | 0.0185 |
| 30.9 | 0.0225 | 0.0225 |
| 25.9 | 0.03 | 0.029 |
| 21.5 | 0.04 | 0.037 |
| 16.1 | 0.05 | 0.05 |

$\mu = 9441$

## TABLE V.

**Blood of Horse 1% (0.9% NaCl). Temperature 16.2°.**

| Amount in c.c. | Hydrochloric acid | Sulphuric acid | Formic acid | Acetic acid | Butyric acid | Oxalic acid | Succinic acid | Lactic acid | Tartaric acid | Ammonia | Sodium hydrate | Potassium hydrate | Lithium hydrate | Amount in c.c. | Barium hydrate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\frac{m}{1}$ | $\frac{m}{2}$ | $\frac{m}{1}$ | $\frac{m}{1}$ | $\frac{m}{1}$ | $\frac{m}{2}$ | $\frac{m}{2}$ | $\frac{m}{1}$ | $\frac{m}{2}$ | $\frac{m}{1}$ | $\frac{m}{1}$ | $\frac{m}{1}$ | $\frac{m}{1}$ | | $\frac{m}{4}$ |
| 0.25 | | | | | | | | | | 60 | | | | 0.5 | |
| 0.2 | | | | 15† | | | | | | | | | | 0.4 | |
| 0.17 | | | | | | | 15 | | | | | | | 0.34 | |
| 0.13 | | | | | | | | | | 120 | | | | 0.26 | |
| 0.1 | | | | | | | | | | | | | | 0.2 | |
| 0.07 | | | | 30§ | | | | 15 | | 210 | 15 | 15 | 15 | 0.14 | 15 |
| 0.05 | | | | 60§ | | | 30 60§ | | 15† | | 30 | 30 | 30 | 0.1 | 30 |
| 0.04 | 15* | | | 120 | | | | 30 | | | 60 | 60 | 60 | 0.08 | 60 |
| 0.03 | | 15† | 15 | | | 15† | 120† | 60 | 30§ | | | | | 0.06 | |
| 0.025 | 30* | 30 | 30 | 450 | | 30 60 | 450† | 120† | 60 | 450† | 120 | 120 | 120 | 0.05 | 120 |
| 0.02 | 60* | 60 | 60 | | | 450 | | 450 | 120 | | 210 | 210 | 210 | 0.04 | |
| | 120§ | 120* | 120† | | | 120§ | | | | | | | 450† | | 210† |
| 0.017 | 450 | 450 | 450 | | | | | | 450 | | 450 | 450 | | 0.034 | 450 |
| 0.013 | | | | | | | | | | | | | | 0.026 | |
| 0.01 | | | | | | | | | | | | | | 0.02 | |
| 0.007 | | | | | | | | | | | | | | 0.014 | |
| 0.005 | | | | | | | | | | | | | | 0 01 | |

* Corresponds to an amount between two that are given, but nearer the larger.
† Corresponds to an amount midway between two that are given.
§ Corresponds to an amount between two that are given but nearer the smaller.

### TABLE VI.

Blood of Horse 2.5% (0.9% NaCl). Tp. 15.2°.

| Amount in c.c | Hydrochloric acid | Sulphuric acid | Acetic acid | Mono- | Di-chlor-acetic acid | Tri- | Oxalic acid | Succinic acid | Lactic acid | Citric acid | Ammonia | Sodium hydrate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\frac{m}{1}$ | $\frac{m}{2}$ | $\frac{m}{1}$ | $\frac{m}{1}$ | $\frac{m}{1}$ | $\frac{m}{1}$ | $\frac{m}{2}$ | $\frac{m}{2}$ | $\frac{m}{1}$ | $\frac{m}{3}$ | $\frac{m}{1}$ | $\frac{m}{1}$ |
| 1.0 | | | 5 15† | | | | | | | | | |
| 0.7 | | | | | | | | | | | 30 | |
| 0.5 | | | | | | | | 15 30 | | | | |
| 0.4 | | | 30 | | | | | | 5 | | 60 | |
| 0.3 | | | | | | | | | | | | |
| 0.25 | | | | | | | | | | 60† | 120 | |
| 0.2 | | | | | | | | | | | | |
| 0.17 | | | | | | | | | | | | 5 |
| 0.13 | | | | | | | 5 | 60 120 | 15 30 | | | |
| 0.1 | 5 15 30 | 5 15 30 | | 5 | | | 15 30 | | | | | 15 |
| 0 07 | | | 60 | 15 | 5 | | | | | | | 30 |
| | | | 120§ | 30 | 15† | 5 15 | | | | 120 | | |
| 0.05 | 120† 60 | 120† 60 | | 120† 60 | 60† 30 | 60† 30 | 60† 120 | | 60 120 | | | 60 |
| 0.04 | | | | | 120 | 120 | | | | | | 120 |
| 0.03 | | | | | | | | | | | | |
| 0.025 | | | | | | | | | | | | |
| 0.02 | | | | | | | | | | | | |
| 0.017 | | | | | | | | | | | | |
| 0.013 | | | | | | | | | | | | |
| 0.01 | | | | | | | | | | | | |
| 0.007 | | | | | | | | | | | | |

For explanation of signs *, †, and § see Table V.

## TABLE VII.

BLOOD OF HORSE 1% (7.8% CANE SUGAR). Tp. 14.6°.

0.9% NaCl.

| Amount in c.c. | Hydrochloric acid $\frac{m}{1}$ | Sulphuric acid $\frac{m}{2}$ | Formic acid $\frac{m}{1}$ | Acetic acid $\frac{m}{1}$ | Di-chlor-acetic acid $\frac{m}{1}$ | Tri-chlor-acetic acid $\frac{m}{1}$ | Butyric acid $\frac{m}{1}$ | Oxalic acid $\frac{m}{2}$ | Succinic acid $\frac{m}{1}$ | Lactic acid $\frac{m}{2}$ | Tartaric acid $\frac{m}{2}$ | Citric acid $\frac{m}{3}$ | Ammonia $\frac{m}{1}$ | Sodium hydrate $\frac{m}{1}$ | Hydrochloric acid $\frac{m}{1}$ | Ammonia $\frac{m}{1}$ | Sodium hydrate $\frac{m}{1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1.5 | | | | | | | | | 5 | | | 14 | | | | | |
| 1.3 | | | | | | | | | 14 | | 5 | | | | | | |
| 1.0 | | | | | | | | | 60† 30 | | 14 | 30 | 90* | 5 | | | |
| 0.7 | | | | | | | | | 90 | | 30 | 60 | | 14 | | | |
| 0.5 | | | | | | | | | 240 | | | 90† | | | | 30 | |
| 0.4 | | | | 5† | | | | | | | 60 | | | 30 | | | |
| 0.3 | | | | 60* 14 | | | | | | | 90 | 240 | | | | 60 | |
| 0.25 | | | | 240 | | | | | | | | | 240§ | 60 | | | |
| 0.2 | | | | | | | | | | 5† 14 | | | | | | 90 | |
| 0.17 | 5 | | | | | | | | | 30* 60 | | | | 90 | | | |
| 0.13 | 14 | | 14† 5 | | | | | | | 90† | 240 | | | | | | 5 |
| 0.1 | | | 60 | | | | | | | | | | | 240§ | | 240 | 14 |
| 0.07 | 30 | 14† | 240† | | | | | 5 14 | | | | | | | 5 | | 30 |
| 0.05 | | 30 | | | | | | 30§ | | | | | | | 14 | | |
| 0.04 | 60 90 | 60 | | | | | | 60 | | | | | | | 30 | | 60 |
| 0.03 | 240 | 90 | | | 5 14 | 30 | | 240 | | | | | | | 60 | | 90* |
| 0.025 | | 240 | | | 90 240 | 240 | | | | | | | | | 90 | | 240 |
| 0.02 | | | | | | | | | | | | | | | | | |
| 0.017 | | | | | | | | | | | | | | | | | |
| 0.013 | | | | | | | | | | | | | | | | | |
| 0.01 | | | | | | | | | | | | | | | | | |
| 0.007 | | | | | | | | | | | | | | | | | |
| 0.005 | | | | | | | | | | | | | | | | | |
| 0.004 | | | | | | | | | | | | | | | | | |
| 0.003 | | | | | | | | | | | | | | | | | |
| 0.0025 | | | | | | | | | | | | | | | | | |
| 0.002 | | | | | | | | | | | | | | | | | |
| 0.0017 | | | | | | | | | | | | | | | | | |

For explanation of signs *, †, and § see Table V.

## TABLE VIII.

### Formic Acid (0.1 *n*).

**10*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 37.0 | 0.17 | 0.16 |
| 34.5 | 0.175 | 0.18 |
| 29.9 | 0.185 | 0.227 |
| 24.5 | 0.32 | 0.3 |
| 20.0 | 0.4 | 0.364 |
| 15.0 | 0.45 | 0.483 |
| 10.45 | 0.6 | 0.62 |

$\mu = 8841$

**20*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 37.0 | 0.13 | 0.128 |
| 34.5 | 0.13 | 0.142 |
| 29.9 | 0.17 | 0.17 |
| 24.5 | 0.21 | 0.214 |
| 20.0 | 0.225 | 0.26 |
| 15.0 | 0.35 | 0.33 |
| 10.45 | 0.4 | 0.405 |

$\mu = 7598$

**30*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 37.0 | 0.13 | [illegible] |
| 34.5 | 0.13 | [illegible] |
| 29.9 | 0.17 | [illegible] |
| 24.5 | 0.185 | [illegible] |
| 20.0 | 0.19 | [illegible] |
| 15.0 | 0.225 | [illegible] |
| 10.45 | 0.25 | [illegible] |

$\mu = 4310$

**40*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 37.0 | 0.13 | 0.132 |
| 34.5 | 0.13 | 0.136 |
| 29.9 | 0.15 | 0.146 |
| 24.5 | 0.17 | 0.157 |
| 20.0 | 0.175 | 0.168 |
| 15.0 | 0.18 | 0.181 |
| 10.45 | 0.19 | 0.195 |

$\mu = 2578$

**50*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 37.0 | 0.13 | 0.128 |
| 34.5 | 0.13 | 0.133 |
| 29.9 | 0.14 | 0.142 |
| 24.5 | 0.16 | 0.156 |
| 20.0 | 0.18 | 0.168 |
| 15.0 | 0.18 | 0.183 |
| 10.45 | 0.185 | 0.198 |

$\mu = 2901$

**180*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 37.0 | 0.12 | 0.12 |
| 34.5 | 0.125 | 0.122 |
| 29.9 | 0.125 | 0.125 |
| 24.5 | 0.125 | 0.129 |
| 20.0 | 0.13 | 0.132 |
| 15.1 | 0.135 | 0.132 |
| 10.4 | 0.14 | 0.14 |

$\mu = 921$

## TABLE IX.

### Acetic Acid. $\frac{1}{1}n$

**10*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 40.2 | 0.025 | 0.0209 |
| 34.7 | 0.035 | 0.0405 |
| 30.1 | 0.065 | 0.073 |
| 24.7 | 0.145 | 0.147 |
| 19.6 | 0.30 | 0.29 |
| 15.1 | 0.45 | 0.532 |

$\mu = 23580$

**15*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 40.2 | 0.02 | 0.0175 |
| 34.7 | 0.03 | 0.033 |
| 30.1 | 0.04 | 0.057 |
| 24.7 | 0.1 | 0.11 |
| 19.6 | 0.225 | 0.212 |
| 15.1 | 0.4 | 0.375 |

$\mu = 22240$

**20*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 40.2 | 0.02 | 0.0152 |
| 34.7 | 0.025 | 0.0251 |
| 30.1 | 0.035 | 0.0364 |
| 24.7 | 0.055 | 0.067 |
| 19.6 | 0.115 | 0.115 |
| 15.1 | 0.25 | 0.183 |
| 10.2 | 0.3 | 0.316 |

$\mu = 18050$

**40*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 40.2 | 0.017 | 0.0131 |
| 34.7 | 0.02 | 0.0203 |
| 30.1 | 0.025 | 0.0298 |
| 24.7 | 0.033 | 0.047 |
| 19.6 | 0.065 | 0.074 |
| 15.1 | 0.13 | 0.11 |
| 10.2 | 0.17 | 0.18 |

$\mu = 15520$

**50*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 40.2 | 0.017 | 0.013 |
| 34.7 | 0.02 | 0.0203 |
| 30.1 | 0.025 | 0.0274 |
| 24.7 | 0.03 | 0.0417 |
| 19.6 | 0.05 | 0.063 |
| 15.1 | 0.1 | 0.091 |
| 10.2 | 0.13 | 0.141 |

$\mu = 14230$

**60*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 40.2 | 0.017 | 0.0114 |
| 34.7 | 0.017 | 0.0164 |
| 30.1 | 0.0225 | 0.0229 |
| 24.7 | 0.03 | 0.0338 |
| 19.6 | 0.04 | 0.0467 |
| 15.1 | 0.075 | 0.07 |
| 10.2 | 0.1 | 0.105 |

$\mu = 13210$

**90*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 40.5 | 0.017 | 0.0134 |
| 35.15 | 0.017 | 0.017 |
| 30.4 | 0.0225 | 0.0213 |
| 24.7 | 0.0285 | 0.0281 |
| 19.6 | 0.035 | 0.0364 |
| 14.5 | 0.04 | 0.047 |
| 10.1 | 0.06 | 0.06 |

$\mu = 8752$

**120*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 35.15 | 0.017 | 0.0164 |
| 30 4 | 0.02 | 0.02 |
| 24.7 | 0.025 | 0.0253 |
| 19.6 | 0.03 | 0.03 |
| 14.5 | 0.033 | 0.0392 |
| 10.1 | 0.05 | 0.0481 |

$\mu = 7459$

**210*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc |
|---|---|---|
| 40.5 | 0.015 | 0.0147 |
| 35.15 | 0.016 | 0.0169 |
| 30.4 | 0.02 | 0.0193 |
| 24.7 | 0.024 | 0.0226 |
| 19.6 | 0.0275 | 0.0262 |
| 14.5 | 0.03 | 0.03 |
| 10.1 | 0.033 | 0.0352 |

$\mu = 5112$

## TABLE X.

### PROPIONIC ACID. $\frac{1}{1}n$.

**10*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 39.15 | 0.0275 | 0.0267 |
| 35.25 | 0.04 | 0.0455 |
| 28.45 | 0.09 | 0.112 |
| 23.6 | 0.275 | 0.35 |
| 19.35 | 0.4 | 0.4 |

$\mu = 24860$

**20*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 39.15 | 0.025 | 0.0176 |
| 35.25 | 0.0275 | 0.0253 |
| 28.45 | 0.04 | 0.0483 |
| 23.6 | 0.07 | 0.079 |
| 19.35 | 0.14 | 0.11 |
| 14.9 | 0.30 | 0.20 |
| 7.8 | 0.40 | 0.44 |

$\mu = 18050$

**30*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 39.2 | 0.02 | 0.0172 |
| 35.15 | 0.022 | 0.0238 |
| 28.45 | 0.04 | 0.042 |
| 23.45 | 0.05 | 0.067 |
| 19.55 | 0.09 | 0.095 |
| 14.8 | 0.20 | 0.149 |
| 7.7 | 0.30 | 0.30 |

$\mu = 15890$

**40*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 39.2 | 0.017 | 0.0152 |
| 35.15 | 0.02 | 0.0207 |
| 28.45 | 0.033 | 0.0353 |
| 23.45 | 0.043 | 0.055 |
| 19.55 | 0.065 | 0.076 |
| 14.8 | 0.15 | 0.116 |
| 7.7 | 0.25 | 0.226 |

$\mu = 15060$

**60*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 39.2 | 0.017 | 0.0116 |
| 35.15 | 0.02 | 0.0153 |
| 28.45 | 0.025 | 0.025 |
| 23.45 | 0.03 | 0.037 |
| 19.55 | 0.04 | 0.0505 |
| 14.8 | 0.06 | 0.078 |
| 7.7 | 0.185 | 0.136 |

$\mu = 13720$

**90*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 39.1 | 0.017 | 0.0164 |
| 35.15 | 0.02 | 0.0192 |
| 28.8 | 0.025 | 0.025 |
| 23.4 | 0.027 | 0.0317 |
| 19.35 | 0.04 | 0.0379 |
| 14.9 | 0.042 | 0.047 |
| 8.0 | 0.085 | 0.0775 |

$\mu = 7690$

**120*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ caic. |
|---|---|---|
| 39.1 | 0.017 | 0.017 |
| 35.15 | 0.02 | 0.02 |
| 28.8 | 0.025 | 0.0227 |
| 23.4 | 0.025 | 0.0265 |
| 19.35 | 0.03 | 0.03 |
| 14.9 | 0.033 | 0.034 |

$u = 5065$

**180*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 39.1 | 0.017 | 0.017 |
| 35.15 | 0.017 | 0.019 |
| 28.8 | 0.0225 | 0.0223 |
| 23.4 | 0.025 | 0.0258 |
| 19.35 | 0.033 | 0.029 |
| 14.9 | 0.035 | 0.033 |
| 8.0 | 0.04 | 0.04 |

$\mu = 4789$

## TABLE XI.

Butyric Acid. $\frac{1}{1}n$.

**10*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 37.0 | 0.03 | 0.03 |
| 34.4 | 0.033 | 0.04 |
| 30.7 | 0.07 | 0.062 |
| 24.35 | 0.13 | 0.13 |
| 19.5 | 0.25 | 0.25 |
| 15.9 | 0.28 | 0.38 |

$\mu = 21550$

**20*m***

| Tp. | $\frac{1}{1}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 34.4 | 0.025 | 0.025 |
| 30.7 | 0.04 | 0.038 |
| 24.35 | 0.065 | 0.076 |
| 19.5 | 0.15 | 0.13 |
| 15.9 | 0.20 | 0.2 |

$\mu = 19890$

**30*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 37.0 | 0.02 | 0.018 |
| 34.4 | 0.0225 | 0.0225 |
| 30.7 | 0.025 | 0.031 |
| 24.35 | 0.048 | 0.052 |
| 19.5 | 0.08 | 0.08 |
| 15.9 | 0.1 | 0.11 |
| 10.3 | 0.2 | 0.19 |

$\mu = 15190$

**40*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 37.0 | 0.018 | 0.016 |
| 34.4 | 0.020 | 0.02 |
| 30.7 | 0.022 | 0.027 |
| 24.35 | 0.03 | 0.043 |
| 19.5 | 0.065 | 0.065 |
| 15.9 | 0.10 | 0.086 |
| 10.3 | 0.13 | 0.14 |

$\mu = 14000$

**50*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 37.0 | 0.017 | 0.016 |
| 34.4 | 0.019 | 0.019 |
| 30.7 | 0.025 | 0.025 |
| 24.35 | 0.028 | 0.04 |
| 19.5 | 0.045 | 0.058 |
| 15.9 | 0.085 | 0.076 |
| 10.3 | 0.12 | 0.12 |

$\mu = 13210$

## TABLE XII.

Maleinic Acid. $\frac{1}{1}n$.

**10*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 37.5 | 0.014 | 0.014 |
| 34.7 | 0.017 | 0.017 |
| 29.9 | 0.03 | 0.0235 |
| 24.7 | 0.04 | 0.034 |
| 19.3 | 0.05 | 0.05 |
| 15.8 | 0.065 | 0.065 |
| 12.2 | 0.07 | 0.086 |

$\mu = 12670$

**20*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 37.5 | 0.014 | 0.013 |
| 34.7 | 0.015 | 0.0145 |
| 29.9 | 0.017 | 0.018 |
| 24.7 | 0.022 | 0.024 |
| 19.3 | 0.03 | 0.032 |
| 15.8 | 0.04 | 0.038 |
| 12.2 | 0.045 | 0.047 |

$\mu = 9118$

**30*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 37.5 | 0.013 | 0.012 |
| 34.7 | 0.013 | 0.013 |
| 29.9 | 0.017 | 0.016 |
| 24.7 | 0.018 | 0.021 |
| 19.3 | 0.025 | 0.027 |
| 15.8 | 0.03 | 0.032 |
| 12.2 | 0.04 | 0.038 |

$\mu = 8281$

## TABLE XIII.

### CITRACONIC ACID. $\frac{1}{1}n$.

| 10m | | | 20m | | | 50m | | |
|---|---|---|---|---|---|---|---|---|
| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. | Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. | Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
| 35 | 0.017 | 0.017 | 35 | 0.013 | 0.012 | 38 | 0.0115 | 0.0115 |
| 30.2 | 0.02 | 0.025 | 30.2 | 0.017 | 0.017 | 35 | 0.013 | 0.012 |
| 23.7 | 0.04 | 0.04 | 23.7 | 0.025 | 0.026 | 30.2 | 0.015 | 0.015 |
| 20.3 | 0.05 | 0.051 | 20.3 | 0.03 | 0.032 | 23.7 | 0.017 | 0.016 |
| 15.6 | 0.085 | 0.076 | 15.6 | 0.047 | 0.045 | 20.1 | 0.017 | 0.017 |
| 9.8 | 0.1 | 0.098 | 9.8 | 0.065 | 0.068 | 15.6 | 0.019 | 0.019 |
| $\mu = 13490$ | | | $\mu = 11690$ | | | $\mu = 4375$ | | |

## TABLE XIV.

### ITACONIC ACID. $\frac{1}{1}n$.

| 15m | | | 20m | | |
|---|---|---|---|---|---|
| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. | Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
| 29.8 | 0.03 | 0.03 | 29.8 | 0.025 | 0.024 |
| 24.2 | 0.05 | 0.051 | 24.2 | 0.0375 | 0.039 |
| 17.8 | 0.085 | 0.096 | 17.8 | 0.05 | 0.069 |
| 14.6 | 0.15 | 0.13 | 14.6 | 0.09 | 0.093 |
| 10.7 | 0.2 | 0.2 | 10.7 | 0.135 | 0.135 |
| $\mu = 17040$ | | | $\mu = 15560$ | | |

## TABLE XV.

### OLEINIC ACID. $\frac{1}{10}n$.

| 10m | | | 22m | | |
|---|---|---|---|---|---|
| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. | Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
| 39.4 | 0.035 | 0.035 | 39.1 | 0.03 | 0.03 |
| 36.7 | 0.06 | 0.05 | 36.2 | 0.035 | 0.042 |
| 31.4 | 0.1 | 0.1 | 31.3 | 0.08 | 0.079 |
| 23.9 | 0.3 | 0.3 | 24.1 | 0.2 | 0.2 |
| $\mu = 25780$ | | | $\mu = 23480$ | | |

## TABLE XVI.

Oleate of Sodium. $\frac{1}{100}$ *n*.

10*m*

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 36.3 | 0.125 | 0.125 |
| 31.4 | 0.14 | 0.14 |
| 24.1 | 0.18 | 0.16 |
| 15.9 | 0.19 | 0.19 |
| 12 | 0.2 | 0.21 |
| 4 | 0.25 | 0.25 |

$\mu = 3776$

## TABLE XVII.

Triolein. $\frac{1}{100}$ *n*.

15*m*

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 38.9 | 0.22 | 0.22 |
| 35.7 | 0.25 | 0.25 |
| 30.9 | 0.27 | 0.36 |
| 24 | 0.6 | 0.6 |

$\mu = 13810$

50*m*

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 38.9 | 0.17 | 0.17 |
| 35.7 | 0.2 | 0.2 |
| 30.9 | 0.32 | 0.26 |
| 24 | 0.4 | 0.4 |

$\mu = 10590$

## TABLE XVIII.

Streptolysin.

20*m*

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 36.1 | 0.08 | 0.08 |
| 31.1 | 0.20 | 0.21 |
| 25.8 | 0.40 | 0.54 |
| 22.9 | 1.30 | 1.15 |

$\mu = 31880$

## TABLE XIX.

Ricin.

c. 30*m*

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 36.2 | 0.05 | 0.05 |
| 27.9 | 0.12 | 0.11 |
| 15.6 | 0.33 | 0.37 |
| 10.1 | 0.70 | 0.66 |
| 0.9 | 1.30 | 1.80 |

$\mu = 17220$

## TABLE XX.

10 *n* $H_2SO_4$.

2% egg albumen.

90*m*

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 35.8 | 0.1 | 0.11 |
| 29.7 | 0.16 | 0.16 |
| 25.4 | 0.225 | 0.21 |
| 19.9 | 0.3 | 0.29 |
| 14.5 | 0.4 | 0.41 |

$\mu = 11050$

## TABLE XXI.

0.1 *n* $HgCl_2$

Red cokpuscles of horse.

45*m*

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 35.8 | 0.085 | 0.085 |
| 30.9 | 0.15 | 0.15 |
| 25.9 | 0.25 | 0.24 |
| 21.9 | 0.55 | 0.36 |
| 16.1 | 0.6 | 0.65 |
| 12.3 | 1.0 | 0.98 |
| 10.8 | 1.0 | 1.15 |

$\mu = 17860$

## TABLE XXII.

### PRECIPITIN.

**73*m***

| Tp. | $\frac{1}{c}$ obs | $\frac{1}{c}$ calc. |
|---|---|---|
| 36.1 | 0.25 | 0.25 |
| 30.1 | 0.3 | 0.306 |
| 20.0 | 0.45 | 0.44 |
| 13.9 | 0.55 | 0.55 |

$\mu = 6284$

## TABLE XXIII.

### COLI-AGGLUTININ.

**20*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 41.2 | 0.0025 | 0.0023 |
| 36.6 | 0.004 | 0.0042 |
| 31.4 | 0.0115 | 0.0086 |
| 25.8 | 0.030 | 0.019 |
| 19.4 | 0.05 | 0.05 |
| 15.6 | 0.06 | 0.092 |
| 12.3 | 0.085 | 0.15 |

$\mu = 25960$

**30*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 41.2 | 0.0007 | 0.00063 |
| 36.6 | 0.001 | 0.0011 |
| 31.4 | 0.001 | 0.0021 |
| 25.8 | 0.0017 | 0.0044 |
| 19.4 | 0.012 | 0.011 |
| 15.6 | 0.018 | 0.018 |
| 12.3 | 0.026 | 0.029 |

$\mu = 23850$

**50*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 41.2 | 0.0004 | 0.00035 |
| 36.6 | 0.0005 | 0.00056 |
| 31.4 | 0.0005 | 0.00098 |
| 25.8 | 0.0007 | 0.00184 |
| 19.4 | 0.002 | 0.0038 |
| 15.6 | 0.0055 | 0.0061 |
| 12.3 | 0.01 | 0.0093 |

$\mu = 20030$

**65*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 40.9 | 0.00018 | 0.000175 |
| 36.7 | 0.0002 | 0.00022 |
| 30.7 | 0.0005 | 0.00038 |
| 25.9 | 0.0005 | 0.00062 |
| 19.4 | 0.0008 | 0.00116 |
| 15.9 | 0.002 | 0.0017 |
| 12.0 | 0.002 | 0.0025 |

$\mu = 17080$

**80*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 41.5 | 0.0002 | 0.000115 |
| 36.7 | 0.00013 | 0.00015 |
| 30.8 | 0.00025 | 0.00023 |
| 25.9 | 0.0002 | 0.00031 |
| 19.5 | 0.00045 | 0.00049 |
| 15.8 | 0.0007 | 0.00064 |
| 12.1 | 0.0017 | 0.00085 |

$\mu = 12200$

**100*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 41.5 | 0.0001 | 0.0001 |
| 36.7 | 0.000085 | 0.000125 |
| 30.8 | 0.000185 | 0.00017 |
| 25.9 | 0.00019 | 0.00022 |
| 19.5 | 0.00035 | 0.00031 |
| 15.8 | 0.0004 | 0.00039 |
| 12.1 | 0.00045 | 0.00047 |

$\mu = 9486$

**120*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 41.9 | 0.000085 | 0.000084 |
| 36.7 | 0.0001 | 0.0001 |
| 30.7 | 0.00018 | 0.00014 |
| 25.9 | 0.0002 | 0.00017 |
| 19.4 | 0.000225 | 0.00023 |
| 15.9 | 0.000275 | 0.00027 |
| 12.0 | 0.00033 | 0.00033 |

$\mu = 8288$

## TABLE XXIV.

### Coli-agglutinin.

| 10m I | | | 10 m II | | | 20m I | | | 20m II | | | 30m | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. | Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. | Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. | Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. | Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
| 38.6 | 0.005 | 0.0036 | 38.6 | 0.005 | 0.005 | 39.5 | 0.0015 | 0.0013 | 39.5 | 0.001 | 0.001 | 38.5 | 0.001 | 0.00088 |
| 34.9 | 0.0055 | 0.0057 | 34.9 | 0.0055 | 0.0088 | 35.7 | 0.002 | 0.002 | 35.7 | 0.0015 | 0.0017 | 35.0 | 0.0012 | 0.0012 |
| 30.9 | 0.01 | 0.011 | 30.9 | 0.02 | 0.016 | 31.5 | | | 31.5 | 0.003 | 0.003 | 30.7 | 0.00175 | 0.0018 |
| 24.0 | 0.04 | 0.035 | 24.0 | 0.05 | 0.05 | 24.1 | 0.01 | 0.0084 | 24.1 | 0.01 | 0.0088 | 24.0 | 0.007 | 0.0034 |
| 21.2 | 0.045 | 0.057 | 21.2 | 0.085 | 0.08 | 20.5 | 0.0125 | 0.013 | 20.5 | 0.007 | 0.015 | 21.6 | 0.007 | 0.0044 |
| 13.2 | 0.25 | 0.25 | 12.7 | 0.35 | 0.35 | 14.1 | 0.03 | 0.039 | 14.1 | 0.04 | 0.041 | 12.7 | 0.01 | 0.011 |
| | | | | | | | | | 10.5 | 0.048 | 0.074 | 9.6 | 0.015 | 0.015 |
| $\mu = 30950$ | | | $\mu = 29340$ | | | $\mu = 22820$ | | | $\mu = 26380$ | | | $\mu = 17180$ | | |

| 40m I | | | 40m II | | | 50m | | | 5h | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. | Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. | Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. | Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
| 38.5 | 0.0007 | 0.0007 | 38.5 | 0.0006 | 0.00065 | 38.5 | 0.0007 | 0.00062 | 38.5 | 0.0002 | 0.00018 |
| 35 | 0.00085 | 0.0012 | 35.0 | 0.00125 | 0.00125 | 35.0 | 0.001 | 0.0011 | 35.0 | 0.0002 | 0.0002 |
| 30.7 | 0.002 | 0.0022 | 30.7 | 0.006 | 0.0037 | 30.7 | 0.002 | 0.0022 | 30.7 | 0.0002 | 0.00024 |
| 24 | 0.008 | 0.0063 | 24.0 | 0.007 | 0.0057 | 24.0 | 0.009 | 0.0069 | 24.0 | 0.0003 | 0.00031 |
| 21.6 | 0.011 | 0.0093 | 21.6 | 0.035 | 0.035 | 21.6 | 0.01 | 0.01 | 21.6 | 0.00042 | 0.00033 |
| 12.7 | 0.04 | 0.04 | 12.7 | 0.04 | 0.044 | | | | 12.7 | 0.00052 | 0.00049 |
| | | | | | | | | | 9.6 | 0.00052 | 0.00055 |
| $\mu = 29030$ | | | $\mu = 28320$ | | | $\mu = 30630$ | | | $\mu = 6907$ | | |

## TABLE XXV.

### B. TYPHOSUS-AGGLUTININ.

**10*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 38.2 | 0.002 | 0.002 |
| 35.2 | 0.006 | 0.0036 |
| 31.1 | 0.007 | 0.0083 |
| 27.4 | 0.02 | 0.018 |
| 19.2 | 0.13 | 0.09 |
| 15.9 | 0.2 | 0.2 |
| 11.5 | 0.5 | 0.55 |

$\mu = 37160$

**40*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 38.6 | 0.0013 | 0.00126 |
| 34.9 | 0.00225 | 0.0024 |
| 19.6 | 0.04 | 0.04 |
| 16.1 | 0.1 | 0.079 |
| 11.4 | 0.15 | 0.2 |

$\mu = 33010$

**120*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 37.8 | 0.0006 | 0.0006 |
| 35.2 | 0.0008 | 0.00079 |
| 31.3 | 0.00115 | 0.00115 |
| 27.4 | 0.0014 | 0.0018 |
| 15.8 | 0.006 | 0.006 |

$\mu = 18690$

**180*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 37.8 | 0.0007 | 0.0007 |
| 35.2 | 0.0009 | 0.00083 |
| 31.3 | 0.001 | 0.00107 |
| 27.4 | 0.0013 | 0.0014 |
| 19.0 | 0.0017 | 0.0025 |
| 11.6 | 0.0045 | 0.0044 |

$\mu = 8511$

## TABLE XXVI.

### VIBRIO-LYSIN.

**20*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 35.3 | 0.17 | 0.1 |
| 29.8 | 0.25 | 0.25 |
| 26.3 | 0.4 | 0.4 |
| 20.6 | 1.0 | 1.0 |

$\mu = 27330$

**40*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc |
|---|---|---|
| 35.3 | 0.115 | 0.078 |
| 29.8 | 0.17 | 0.17 |
| 26.3 | 0.22 | 0.27 |
| 20.6 | 0.6 | 0.6 |

$\mu = 24410$

**100*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 40.1 | 0.0275 | 0.0275 |
| 35.3 | 0.05 | 0.044 |
| 29.5 | 0.07 | 0.08 |
| 26.0 | 0.1 | 0.117 |
| 20.4 | 0.25 | 0.215 |
| 16.1 | 0.42 | 0.394 |

$\mu = 19340$

**180*m***

| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
|---|---|---|
| 39.8 | 0.03 | 0.03 |
| 35.4 | 0.042 | 0.043 |
| 29.3 | 0.07 | 0.073 |
| 25.3 | 0.1 | 0.104 |
| 20.1 | 0.17 | 0.17 |
| 15.6 | 0.3 | 0.25 |

$\mu = 15840$

## TABLE XXVII.

### TETANOLYSIN.

| **60m** | | | **65m** | | |
|---|---|---|---|---|---|
| Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. | Tp. | $\frac{1}{c}$ obs. | $\frac{1}{c}$ calc. |
| 30.4 | 0.4 | 0.37 | 35.3 | 0.45 | 0.45 |
| 25.6 | 0.5 | 0.53 | 31.1 | 0.58 | 0.58 |
| 21.9 | 0.8 | 0.66 | 24.9 | 0.8 | 0.83 |
| 15.4 | 1.0 | 1.0 | 10.8 | 2.0 | 2.1 |
| 12.1 | 1.3 | 1.27 | | | |
| $\mu$ = 10860 | | | $\mu$ = 10860 | | |

## TABLE XXVIII.

| | | |
|---|---|---|
| 1.0 c.c. | 0.1 c.c. | 0.01 c.c. |
| 0.7 | 0.07 | 0.007 |
| 0.5 | 0.05 | 0.005 |
| 0.4 | 0.04 | 0.004 |
| 0.35 | 0.035 | 0.0035 |
| 0.3 | 0.03 | 0.003 |
| 0.25 | 0.025 | 0.0025 |
| 0.2 | 0.02 | |
| 0.17 | 0.017 | |
| 0.13 | 0.013 | |

## TABLE XXIX.

### TETANOLYSIN.

| Amount in c.c. | 40° | 26° | 16° | 9° |
|---|---|---|---|---|
| 1.3 | | | | $H_{10}$ |
| 1.0 | | | | |
| 0.8 | $H_{10}$ | | | |
| 0.6 | | | | |
| 0.5 | $H_{10}$ | | | |
| 0.4 | | | | $H_{20}$ |
| 0.3 | $H_{10}$ | | | |
| 0.25 | | $H_{10}$ | $H_{10}$ | |
| 0.2 | $H_{10}$ | | | $H_{40}$ |
| 0.17 | | | | |
| 0.13 | | | | |
| 0.1 | | | $H_{20}$ | |
| 0.08 | $H_{10}$ | | | |
| 0.06 | | | $H_{40}$ | |
| 0.05 | | | | |
| 0.04 | $H_{10}$ | | | |
| 0.03 | | | | |
| 0.02 | | | | |
| 0.015 | | | | |

## TABLE XXX.

### TETANOLYSIN.

**25m**

| Tp. | $\frac{1}{c}$ |
|---|---|
| 41.7 | 0.25 |
| 36.5 | 0.6 |
| 31.7 | 0.03 |
| 25.7 | 0.07<br>or 0.2 |
| 21.2 | 0.17 |
| 14.9 | 0.2 |
| 6.7 | 0.4<br>or 0.8 |

## TABLE XXXI.

| Tp. | 10m | 10m | 20m | 40m | 60m | 90m | 120m |
|---|---|---|---|---|---|---|---|
| | $\frac{1}{c}$ | $\frac{1}{c}$ | $\frac{1}{c}$ | $\frac{1}{c}$ | $\frac{1}{c}$ | $\frac{1}{c}$ | $\frac{1}{c}$ |
| 41.7 | 0.35 | 0.4 | 0.185 | 0.1 | 0.07 | 0.05 | 0.06 |
| 36.5 | 0.55 | 0.5 | 0.2 | 0.1 | 0.07 | 0.07 | 0.045 |
| 31.7[1] | 0.25<br>or 0.06 | 0.17<br>or 0.025 | <0.03 | 0.025 | 0.0275 | 0.017 | 0.013 |
| 25.7 | | 0.6 | 0.225 | 0.07 | 0.085 | 0.085 | 0.0275 |
| 21.2 | 0.8 | | 0.1 | 0.05 | | 0.06 | 0.04 |
| 14.9 | 0.18 | | 0.07 | | 0.085 | 0.06 | 0.07 |
| 6.7 | 1.7 | 2.0 | 0.4 | 0.17 | 0.115 | 0.2 | 0.085 |

## TABLE XXXII.

### TETANOLYSIN.

Heating at 37°.

| 24/2 | | 25/2 | | 26/2 | | 27/2 | | 1/3 | | 3/3 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| t | $\frac{1}{c}$ | t | $\frac{1}{c}$ | t | $\frac{1}{c}$ | t | $\frac{1}{c}$ | t | $\frac{1}{c}$ | t | $\frac{1}{c}$ |
| 0m | 0.5 | 0m | 0.7 | 0m | 0.225 | 0m | | 0m | 0.2 | 0m | 0.07 |
| 30 | 0.45 | 30 | 0.5 | 20 | 0.2 | 5 | 0.03 | 5 | 0.175 | 10 | 0.07 |
| 60 | 0.275 | 60 | <0.1 | 30 | 0.03 | 10 | 0.05 | 10 | 0.15 | 20 | 0.07 |
| 120 | <0.2 | 120 | 0.7 | 40 | 0.33 | 20 | 0.05 | 20 | 0.1 | 30 | 0.05 |
| | | 180 | 0.7 | 50 | 0.5 | 30 | 0.05 | 40 | 0.1 | 40 | 0.03 |
| | | 240 | 0.7 | 60 | 0.5 | 40 | 0.07 | | | 50 | 0.03 |
| | | | | 80 | 0.7 | 50 | 0.4 | | | 60 | 0.07 |
| | | | | 100 | 0.55 | 60 | 0.35 | | | 90 | 0.1 |
| | | | | 120 | 0.7 | 80 | 0.3 | | | 120 | 0.1 |
| | | | | 180 | 0.5 | 120 | 0.3 | | | 180 | 0.16 |
| | | | | 240 | 0.225 | 240 | 0.3 | | | 240 | 0.13 |
| | | | | 360 | 0.3 | 360 | 0.3 | | | 300 | 0.06 |
| | | | | | | 480 | 0.3 | | | 360 | 0.04 |
| | | | | | | 600 | 0.3 | | | 420 | <0.02 |
| | | | | | | | | | | 480 | <0.02 |

## TABLE XXXVI.

### NAJA TRIPUDIANS.

| | 5m $\frac{1}{c}$ obs. | | | 10m $\frac{1}{c}$ obs. | | | 15m $\frac{1}{c}$ obs. | |
|---|---|---|---|---|---|---|---|---|
| Tp. | I | II | Tp. | I | II | Tp. | I | II |
| 37.3 | 0.4 | 0.3 | 37.3 | 0.275 | 0.225 | 37.3 | 0.275 | 0.225 |
| 35.3 | 0.4 | 0.3 | 35.3 | 0.3 | 0.25 | 35.3 | 0.275 | 0.225 |
| 30.8 | 0.4 | 0.33 | 30.8 | 0.35 | 0.26 | 30.8 | 0.3 | 0.25 |
| 24.1 | 0.5 | 0.33 | 24.1 | 0.4 | 0.35 | 24.1 | 0.4 | 0.3 |
| 17.7 | 0.4 | 0.3 | 17.7 | 0.45 | 0.35 | 17.7 | 0.45 | 0.3 |
| 14.2 | 0.6 | 0.45 | 14.2 | 0.5 | 0.38 | 14.2 | 0.35 | 0.25 |
| 10.8 | 0.5 | 0.4 | 10.8 | 0.45 | 0.3 | 10.8 | 0.3 | 0.25 |

## TABLE XXXVII.

### ANCISTRODON PISCIVORUS.

| | 5m $\frac{1}{c}$ obs. | | | 10m $\frac{1}{c}$ obs. | | | 15m $\frac{1}{c}$ obs. | |
|---|---|---|---|---|---|---|---|---|
| Tp. | I | II | Tp. | I | II | Tp. | I | II |
| 39.3 | 0.5 | 0.38 | 39.3 | 0.4 | 0.35 | 39.3 | 0.4 | 0.28 |
| 35.2 | 0.5 | 0.36 | 35.2 | 0.45 | 0.35 | 35.2 | 0.4 | 0.28 |
| 30.7 | 0.4 | 0.35 | 30.7 | 0.42 | 0.35 | 30.7 | 0.4 | 0.3 |
| 28.2 | 0.4 | 0.35 | 28.2 | 0.4 | 0.35 | 28.2 | 0.45 | 0.33 |
| 19.2 | 0.4 | 0.3 | 19.2 | 0.35 | 0.28 | 19.2 | 0.4 | 0.3 |
| 14.8 | 0.4 | 0.3 | 14.8 | 0.35 | 0.28 | 14.8 | 0.3 | 0.25 |
| 10.6 | 0.4 | 0.25 | 10.6 | 0.3 | 0.25 | 10.6 | 0.25 | 0.225 |

# THE FORMATION OF TRUE BONE WITH CELLULAR (RED) MARROW IN A SCLEROTIC AORTA.

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At a recent post-mortem examination in the Johns Hopkins Hospital one of the most striking lesions found was an extremely sclerotic and calcified aorta. Microscopical sections from the dorsal portion of this aorta showed at one point in the much thickened intima a new formation of true bone. While the finding is not unparalleled, the references in medical literature to similar lesions are so few that the case seems worthy of note, especially as in one particular at least this case differs from those already reported.

The patient was a white male 72 years old, and was admitted to the surgical service for the removal of a hypertrophied prostate, but died before the operation, apparently as the result of a general infection, following an attack of furunculosis, and of complications as indicated in the anatomical diagnosis. The post-mortem examination was made by the author August 12, 1904, three hours after death. It does not seem necessary for the purpose of this paper to give the full post-mortem findings. The Anatomical Diagnosis was as follows:

*Hypertrophy (adenomyoma) of the prostate; dilatation of the bladder with hypertrophy of its musculature; chronic diffuse nephritis; aortic sclerosis with calcification; furunculosis; purulent bronchitis; broncho-pneumonia; mural thrombi of aorta; embolism and thrombosis of the right brachial artery, with gangrene of the right extremity, and of the right internal carotid artery, with encephalomalacia of the right cerebral hemisphere; atrophic spleen; surface atrophy of the liver.*

The lesions of interest are those of the aorta and its branches. In addition to the sclerosis, the presence of the thrombi in the aorta seems noteworthy. The larger of these formed a globular mass about 15 millimetres in diameter, attached to the convex side of the ascending portion of the aorta at a point just below the orifice of the innominate artery. It was firmly adherent to the wall of the vessel. This was quite evidently the source of the emboli which were carried to the right brachial and carotid arteries. A smaller, oval, somewhat flattened thrombus mass capped a roughened, projecting, calcified plaque in the upper portion of the thoracic aorta.

The whole aorta showed extreme sclerosis with calcified plaques throughout its extent. The calcification, however, was most marked in the lower dorsal portion and just above its bifurcation the aorta was a rigid tube with walls of bony hardness. It was through this portion that microscopical sections were made after decalcification in 5 per cent. nitric acid.

The sections gave the following picture on microscopical examination:

The adventitia appears normal, showing no increase in the density of the fibrous tissue. At the ends of the section the media is considerably thinner than normal and is about one third as wide as the thickened intima. The inner portion of the media (that next the intima) has a denser appearance than the outer part. Here its fibres stain more deeply with eosin and are in part of the section distinctly hyaline, with but here and there a shrunken pyknotic nucleus. Throughout the media the nuclei seem to be reduced in number. Toward the middle part of the section the media becomes even thinner, the hyaline degeneration of the inner portion is more extensive, and in many places there has been a deposition of calcium salts in the hyaline area. At the thinnest part of the media, its continuity is almost completely interrupted by the passage obliquely through it of vessels of considerable size, surrounded by granulation tissue, which extends inward into the intima. At this point the muscle fibres are extremely atrophied and the nuclei much shrunken.

The intima is thickened about the whole circumference of the aorta. The thickening is irregular with numerous nodules projecting toward the lumen, the largest of which lies over the point in the media where the muscle fibres are most atrophied. This nodule is of the nature of the so-called atheromatous abscess. Toward the lumen of the aorta there is a fairly well-preserved layer of dense connective tissue, poor in cells. This is in large part hyaline and toward the centre of the nodule shows deposits of calcium salts deeply stained by the hæmatoxylin. The main mass of the nodule is made up of a granular debris, in which are calcium salts and numerous slit-like spaces evidently left by the solution of cholesterin crystals. There are also a few round vacuoles, evidently fat spaces. There has been at one point toward the media an attempt at organation and repair. One finds here fairly well-formed vascular connective tissue, n which are numerous lymphoid cells as well as larger mononuclear cells which contain as a rule coarse granules of a light yellow pigment. There are in this part of the intima masses of deep red (eosin staining) refractile materail, often surrounded by cells, which are evidently deposits of old hyaline fibrin.

At the base of this atheromatous nodule and lying directly adjacent to the calcified media are two masses of true bone, not connected with each other in any of the sections studied, though lying quite close together. The larger mass is ring-shaped and has a definite marrow cavity. The bone gives indication of a definite laminar structure. It contains numerous typical bone-corpuscles and is penetrated at its thickest portion by a capillary vessel surrounded by a layer of cells resembling osteoblasts closely applied to the wall of bone, the whole appearing to represent a Haversian canal. About this mass of bone there is a layer of cells resembling osteoblasts and forming a definite membrane, while at many places in the sections studied are multinuclear cells of the typical osteoblast type. A few of these lie in lacunar depressions in the bone but many more are found in similar depressions in the adjacent calcified media. The marrow cavity of the bone contains in a delicate reticulum numerous fat cells and between these a few cells of the marrow type; large mononuclear cells, among which a few are phagocytic and contain yellow blood pigment; small mononuclear and polymorphonuclear cells, both neutrophilic and eosinophilic. This marrow, however, is not so cellular as the portion lying between this ring of bone and the second bone mass. This second mass consists of two bars, a slender one lying immediately adjacent to the calcified media and a larger one separated from the first by a small amount of myeloid tissue, containing vessels, fat cells, and large multinuclear giant cells, apposed to the media. These masses of bone are also surrounded by a layer of osteoblasts and contain bone corpuscles.

The tissue lying between these bony masses has the typical appearance of the cellular red marrow of cancellous bone. It is quite richly vascularized by delicately walled capillaries. There are scattered fat cells in the area, and all the types of cells usually found in red marrow, with a tendency to the grouping normally found there. Under the low power one is struck first by the numerous giant cells of the megalokaryocyte type with large twisted polymorphous nuclei. Under higher power one is able to demonstrate mononuclear cells of the myelocyte type, many of which contain eosinophile granules, polymorphonuclear cells both eosinophilic and neutrophilic, lymphoid cells and cells of the erythrocyte series, non-nucleated red cells, normoblasts with their pyknotic nuclei and hæmoglobin-containing cell bodies with sharp contour, and similar cells but with reticular nuclei.

The picture then in brief is that of an aorta with a media much atrophied and showing hyaline degeneration and in places the deposition of calcium salts. The intima is much thickened, shows atheromatous degeneration, and in places the signs either of trauma and hemorrhage or of an inflammatory process (old hyaline fibrin masses and blood pigment in phagocytes). There is also invasion of the intima by vessels from the adventitia surrounded by young connective tissue cells and mononuclear cells, possibly an attempt at organization following the hemorrhage or inflammation. Where this tissue comes in touch with the calcified tissue there is the formation of true bone with a cellular marrow as found in cancellous bone.

The cases reported in medical literature of bone formation in sclerotic vessels are not numerous, as has been said in a preceding paragraph. Of those reported but six concern the aorta, the two earliest of which were described by Mönckeberg.[1] Marchand[2] in his article in Eulenburg's *Real-Encyclopädie* described a case in which in the crural artery he found the calcified media invaded by new vessels with the absorption of the calcified material and the deposition of the bone by osteoblasts upon the calcium deposits with the formation of a marrow space around the vessel.

Howse[3] in 1877 described a focus of bone in the axillary artery. The axillary artery of the patient had been ruptured by trauma and at his death some time subsequently the artery was found surrounded at the point of injury by a firm mass of connective tissue, in which there was found microscopically cancellous bone "produced in tissue which represents and springs from an altered condition of the middle coat."

In 1886 Paul[4] demonstrated before the London Pathological Society a specimen from a sclerotic tibial artery in which there was a focus of bone formation in the intima.

In the same year Cohn[5] investigated a number of calcified areas in the aorta, without finding anything "even approaching the picture of a bone corpuscle," but did find in a sclerotic and calcified aortic valve a mass of true bone adjacent to the calcified material; and also three cases in which there was bone formation in the media of the crural artery.

In 1900 Rosenstein[6] reported bone formation in one calcified aortic segment, and both bone and cartilage in another leaflet of the same valve.

Rohmer[7] followed in 1901 with three cases, one with bone in the aortic valve, one in the mitral, and one in which there were found several foci of bone in the intima of the femoral artery.

v. Schrötter[8] in 1901 described bone in an organized thrombus in the anterior tibial artery and also in another case in the intima of the crural artery.

The most extensive search for bone formation in sclerosed vessels has been made by Mönckeberg,[1] who in 1902 reported ten successful cases in the study of about 100 cases. In two cases he found bone both in the intima of the aorta and in the media of the femoral artery, in one in the media of the femoral, in one in both media and intima of the femoral, in one case in the intima of the tibialis posticus, and one in the intima and media of both the tibialis anticus and posticus. In this latter case cartilage was formed. He adds in a note to his article that in addition to these six cases he has been successful in four others in finding bone in medium-sized sclerotic vessels, but does not give any further details.

**Thorel**[9] has found bone formation in the aorta and in the femoral and tibial arteries.

**Poscharissky**[10] examined fourteen hearts and found bone in three of them, twice in the mitral valve and once in the calcified annulus of the left venous ostium. Of thirty-one vessels examined, four gave positive results, three of which were aortæ, the fourth being the femoral artery. He records further the finding of vascular granulation tissue in vessels in five autopsies.

In all then, bone formation has been found in the aorta six times, in the intima and media of medium-sized arteries twenty-two times, and in heart valves eight times. This cannot represent the frequency of the occurrence of the condition. Virchow[11] had noted the lesion and in his *Cellularpathologie* stated emphatically that, although most of the so-called bony aortæ and vessels were undoubtedly calcified, the formation of true bone does occur, although he cited no specific case. Orth[12] similarly describes in his text-book the formation of bone in the intima of the aorta, again without reference to any special case. Although the author has made no investigation of the frequency of the occurrence of the lesion, Mönckeberg's figures of ten positive results in a hundred cases of marked sclerosis would indicate that the lesion is regarded as rare chiefly because it is not more often searched for.

For an explanation of the presence of these foci of bone in such unexpected places, one would turn naturally to the Cohnheim doctrine of embryonic displacement of tissue, were not the cases so numerous, and did not the figures just quoted show such a frequency of occurrence of the process in advanced arteriosclerosis.

Another argument against such a theory is the evidence offered by the investigation of Pollack,[13] under Lubarsch's direction, of calcified foci in the lungs, as a result of which, of forty-seven cases studied, free bone was found adjacent to the calcium deposits in forty-three. Poscharissky likewise has conducted an extensive search for bone formation in calcified foci in various organs and records seventeen positive findings in the lungs of twenty-eight cases, two in the tonsils, four positive in the lymph glands, twelve in the dura mater, one in a calcified myoma uteri, two positive and one negative in the liver, one in an adrenal tumor, and one in a hypernephroma of the kidney, besides those in the heart and vessels quoted above.

The final and convincing evidence against the metastasis or displacement theory is afforded by the experimental work of Barth,[14] of Sacerdotti and Frattin, [15] and of Poscharissky.[illegible] Barth introduced a portion of incinerated bone into the peritoneal cavity of a cat. Six weeks later it was found penetrated by connective tissue and in several places there was found true bone in contact with the calcium-containing material, lined by osteoblasts arranged partly in a regular row and partly in groups, forming an osteoid tissue which passed over without a sharp line into ordinary connective tissue.

Sacerdotti and Frattin tied off the renal vessels on one side in four rabbits. Three months later the animals were killed and in three of them there was found a formation of true bone adjacent to the calcium deposits in the necrotic tissue, with a growth of vascular granulation tissue into the kidney from the capsule. This experiment was repeated by Poscharissky on five rabbits with successful results in three animals. Similar experiments by him on the spleen and ovary in the rabbit were unsuccessful, obviously because there was no capsule in these organs with independent circulation from which granulation tissue might grow.

It seems clear, then, that one has here a metaplasia of connective tissue cells into osteoblasts, for in vessels and heart valves there can be no possibility of periosteal or perichondral sprouts playing a part, although in pulmonary foci such a possibility is not so easily excluded. As to the stimulus which leads to this metaplasia, there seems to be a uniformity of opinion as might be expected from the similarity of the pictures presented by the different cases. The process was clearly stated by Paul as consisting of three steps:

(1) Calcareous degeneration.

(2) Irritation about these places from fracture or other injury leading to inflammatory proliferation.

(3) Ossification in this young proliferating tissue.

In all the cases the bone has been formed where young connective tissue has come into contact with calcium deposits. The metaplasia is apparently the result of the chemical stimulus of the calcium salts. These two factors are accepted by all, but there still seems some question as to the cause for the invasion of the intima of the sclerotic arteries by the new vessels and connective tissue. Paul suggested trauma of the nature of fracture of the calcified plates. This view is supported by Howse's case of rupture of the axillary artery; by one of Cohn's cases, in which there was a rupture of the calcified media of the crural artery; and possibly by the present case, in which there were signs of hemorrhage and old inflammatory exudate in the intima of the aorta. Poscharissky insists that in addition to the calcium salts there is always a deposit between the granules of a material resembling amyloid.

The question as to the mode of the formation of the bone I shall not discuss at length. There is about an equal division of those who have written on the subject between the two views:

(1) That there is a direct metaplasia of connective tissue into bone after the manner of callus formation, and

(2) That the calcified material is eroded with the formation of vascularized spaces containing young connective tissue cells, some of which take on the function of osteoblasts and lay down bone, some of the osteoblasts becoming included and forming the bone corpuscles.

The different cases give evidence that the bone may be formed in either way. In the present case the bone would seem to have been formed by the second method. There was nothing in the section suggesting the formation first of osteoid tissue, then of bone, but instead one found erosion of calcified material being carried on by osteoclasts. There were marrow spaces containing capillary loops, and osteoblasts arranged in a row or membrane adjacent to the bone. The picture was quite that seen at the epiphyseal line, with, however, the calcified media of the aorta in place of the calcified cartilage of the epiphysis. The determination of the exact mode of formation of the bone does not seem of so much interest as is the bare fact that in a person of advanced years the connective tissue cells under the stimulus of the calcium salts should retain the power of differentiation into osteoblasts. The patient in this case was 72 years of age. The six of Mönckeberg's cases in which the age was specified were between 62 and 84 years. The greater number of Lubarsch's cases were in patients of advanced age, although there were seven under 45 years and one under 10. Poscharissky obtained only negative results in calcified lung foci in four persons under 28 years.

The present case differs from all others thus far reported in which bone was found in the vessels, in the nature of the marrow found in the intima of the aorta adjacent to the bone. In the previous cases the marrow when present was fatty. In this there was true cellular marrow indistinguishable in section from the marrow of the ribs or of the cancellous bone. Attention was first attracted to it by the rather numerous giant cells with large twisted nuclei distinctly of the megalokaryocytic type. Closer study showed the presence of myelocytes and polymorphonuclear cells, both eosinophilic and neutrophilic, mononuclear and lymphoid cells, and cells of the erythrogenetic group; nucleated cells of the normoblast type and others showing reticular nuclei, as well as many erythrocytes.

The origin of this marrow and its relation to the surrounding tissue seem of the greatest interest. If one excludes the possibility that the bone-formation represents an embryonic displacement, as I think has been done, that explanation can hardly apply to the marrow, and one is left with but two possible explanations: (1) that one has in this also a direct metaplasia of the granulation tissue cells, or (2) that the tissue is the result of transportation of bone marrow cells by the blood stream and their emigration from the blood vessels. Let us consider the latter proposition first. If we accept this view and regard the marrow tissue as the result of emigration of cells we are forced to accept either one of two further propositions. If one be a believer in the Ehrlich idea that in adult life there is no genetic connection between the various types of leucocytes, the granular and the mononuclear (hyaline) groups, or between them and the erythrocytic group, to maintain the emigration idea in this case he must postulate the emigration of a parent cell for each one of the several types found. This seems on the face of it improbable, as the myelocytes, nucleated red cells, and giant cells are so infrequently found in the circulation in general, and possess so little migratory power. It would seem that one might disregard this possibility as being too remote. The other possibility which is open, if one accepts the emigration theory, is that there has occurred the outwandering of a cell or of cells of an indifferent type, which are capable of differentiating into the various forms of blood cells. The view that such a cell exists in the marrow seems to be gaining adherents among students of hematology, as the result of the writings of Pappenheim, Wolff, Hirschfeld, Naegeli, and others. This cell is a simple undifferentiated cell with large round vesicular nucleus and scanty protoplasm and has been variously called, the large lymphocyte, the indifferent lymphoid cell, the myeloblast, Troje's *Markzell*, etc. Such a cell is found in the cell groups of the marrow and in the germinative centres of the lymph glands. It is found in the circulatory blood under certain conditions, in greatest numbers in acute lymphocytic leukæmia. By an increase in its protoplasm it becomes the large mononuclear cell always found in the circulation. If one grants that such a cell is capable of differentiation into the various types of blood cells in adult life, as is strenuously denied by the Ehrlich school, and as strenuously maintained by others, there is no great difficulty in explaining the marrow focus found in the aorta in question.

It would seem more rational, probably, to assume that a single process was operative in the formation of both the bone and the marrow, that is that they were both the result of metaplasia of the connective tissue cells. The bone corpuscles and marrow cells have a common embryonic ancestor in the undifferentiated mesoblastic cell, although in adult life they seem to have diverged widely in nature and function. While it seems but a short step from fibroblast to osteoblast, merely a question of the nature of the intercellular substance laid down, it seems a much greater transition from the fibroblast to the polymorphonuclear cell with its specific granulation, its highly developed power of motility, and its power of phagocytosis. Yet it appears (Jackson) that the cells of the marrow in the fœtus are developed from the cells of the original invading periosteal bud, the osteoblasts. So in this case the same stimulus which led to the metaplasia of connective tissue cells into osteoblasts may have determined their further differentiation into marrow cells, or possibly the stimulus may have been the presence of the newly formed bone.

Although this is the only case recorded in which marrow of this type has been found in the blood vessels, it has been found adjacent to bone foci in other organs. Pollack says that whenever bone is formed in the lung, marrow is also formed, though usually it is of the fatty type but showing a few lymphoid cells and cells of myelocytic type. In two cases he found also nucleated red cells. Poscharissky found marrow in every case, but his result is to be accepted only if one accepts his definition of marrow, for he considers the cellular granulation tissue described in every case marrow, and says that usually in old cases it becomes fatty marrow. He also, however, found in some cases erythroblasts, megalokaryocytes, osteoblasts, and myelocytes. Gierke [16] has also described in a calcified adrenal in a 3½-year-old girl, the formation of bone with a marrow focus containing giant cells, myelocytes, and polymorphonuclear cells and eosinophiles.

The case then here reported is not unparalleled, and it would seem that one must accept the view that the marrow focus was the result of metaplasia of cells of the young granulation tissue in the environment of the newly-formed bone. There is but one point which prevents these cases from being absolutely positive evidence of the formation of blood cells from connective tissue cells in adult life, and that is the presence in young granulation tissue of round cells, mononuclear cells of doubtful antecedents but identical in form and staining characteristics with cells of the blood and marrow. Pappenheim, among others, insists that the whole group of round cells and plasma cells found in granulation tissue are histogenetic (as opposed to hæmatogenetic) in origin, but emphasizes in a recent communication the fact that indifferent lymphoid cells (histogenetic) are found in every granulation tissue. The extensive and careful researches of Maximow led him to the conclusion that the cells of the mononuclear group found in granulation tissue were emigrated cells from the blood stream, and thus arise either from marrow or lymph-glands, and for this view there seems much evidence. The embryonic type of these cells and their similarity to if not identity with cells of the blood and marrow would suggest that the marrow arose from a metaplasia of them, rather than of the fixed connective tissue cells of adult type, and upon the settling of the question of their origin depends the decision in the case here recorded as to whether the marrow focus is the result of metaplasia of connective tissue cells or of metaplasia of indifferent cells of lymphoid type which have emigrated from the blood stream. However that question may be decided, the cases do show that cells of the connective tissue and blood groups retain into late life embryonic characteristics and are capable of a diverse development under appropriate stimuli, and further that there is a much closer genetic relation between the types of blood cells in adult life than is generally accepted.

I would conclude from the study of this case and the literature bearing on the subject, that:

(1) Metaplastic formation of bone in sclerotic vessels is not a rare phenomenon.

(2) The factors in its formation are extensive sclerosis with the presence of calcium deposits; traumatic or inflammatory disturbance in the calcified area, with penetration of the same by granulation tissue; the formation of bone adjacent to the calcified areas.

(3) The bone may be deposited in two ways: either by the conversion of connective tissue into a callus or osteoid tissue with subsequent conversion into bone and at times with the formation of cartilage; or by the resorption of calcified material by vessels and osteoclasts, the formation of a marrow space, and the deposit of bone in its periphery by an osteoblastic membrane derived by metaplasia from the connective tissue cells accompanying the vessel.

(4) In the present case, in which all of the elements of true

cellular marrow were found, one is not justified in deciding whether they were formed by a further metaplasia of connective tissue cells or by the metaplasia of emigrated cells from the blood stream, capable of differentiation into the various types of marrow cells.

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# THE RELATION OF TYPES OF DIARRHŒA IN CHILDREN TO STRAINS OF BACILLUS DYSENTERIÆ.[1]

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The association of the dysentery bacillus with diarrhœa in infants is now established. Previous studies on the subject from this laboratory having proven this occurrence, we undertook as our present thesis the separation from the dejecta of all bacteria which could be cultivated, and which agglutiinated with the patient's blood, exhibited pathogenic properties for laboratory animals, or belonged to the so-called dysentery group of bacilli. A part of our problem, to which we gave particular attention, was the determination of the special type, strain, or variety of dysentery bacilli occurring in the different classes of cases of diarrhœa, and the frequency with which these strains were found associated with the cases.

The entire number of infants studied was seventy-four. They could be divided clinically into the several groups which follow with the number of cases of each group studied.

Inflammatory colitis,—29 cases.
Toxic, dyspeptic, or fermental diarrhœa,—36 cases.
Intestinal indigestion,—7 cases.
Chronic gastro-enteritis,—1 case.
Tuberculous ileo-colitis,—1 case.

In twenty of the cases malnutrition was a marked feature. It was thought unwise to attempt further to subdivide the cases. It is doubtful if our series included any of the nervous diarrhœas given in the classification suggested by Dunn.[2] The result of treatment was as follows: Thirty children recovered completely, twelve improved, one was unimproved, and thirty-one died.

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[1] This study was carried out with the aid of a grant from the Rockefeller Institute for Medical Research.

[2] Dunn, *Archives of Pediatrics*, 1905, xxii, 401.

In order that a comparison could be made between the types of clinical disease and the bacteriological findings this series of cases was further subdivided according to the results of the bacteriological studies. The subdivisions employed were the following:

A. Cases in which the Shiga type of dysentery bacillus was alone found.

B. Cases in which the Hiss' Bacillus Y dysentery organism was found.

C. Cases in which the Flexner-Manila bacillus was the only type isolated.

D. Cases in which several members of the dysentery bacillus group were present.

E. Cases in which the variety of the dysentery bacillus present was not determined.

F. Cases in which a lactose-fermenting organism of the "dysentery-like" group was the only pathogenic form isolated.

G. Cases in which pathogenic streptococci were present in addition to some members of the dysentery group.

H. Cases in which streptococci were the only pathogenic organisms found.

I. Cases in which other organisms than dysentery bacilli and streptococci, pathogenic for animals and agglutinating with the patient's blood, were found.

J. Cases with negative special bacteriological findings.

In group A, in which the Shiga type of dysentery bacillus was present to the exclusion of other pathogenic species on aerobic plates, there were four cases. Of these four children three were admitted as instances of malnutrition, to which condition, it was believed, the intestinal infection was added later. The remaining child had high fever on admission. In view of the recent statement of Charlton and Jehle[3] that children infected with the Shiga type of bacillus show more severe and acute symptoms than children infected with the Flexner type, these cases are important.

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[3] Charlton and Jehle, *Transactions of the Association of American Physicians*, 1904, xix, 405.

It may be mentioned here that they do not bear out this general statement.

In all these children the bowel derangement began with diarrhœa and mucous stools. In only one child was blood and pus noted. Nausea occurred in two infants. All the cases occurred in July. Two infants recovered, one died, and one on discharge was unimproved. At the autopsy in the fatal case patches of congestion and swelling of the lymphoid structures in the intestine only were found.

The identification of the Shiga bacillus was complete. Further reference to its properties will be made in the more strictly bacteriological part of this paper.

In group B, or the cases in which the type of dysentery organism isolated was the so-called "Y" bacillus, there occurred fourteen cases. Six of these belong to the dyspeptic diarrhœa clinical group, six to the inflammatory ileo-colitides, and the remaining two cases were diagnosed intestinal indigestion. In five children of this series (two with dyspeptic diarrhœa and three with ileo-colitis) marked malnutrition preceded the onset of acute symptoms. Seven of the children were rickety. The stools all showed mucus, and in five cases blood, mucus, and pus were mixed together. The quantity of blood was never large. These patients were often very ill; eight were profoundly toxic and five almost or really collapsed on admission. The temperatures were irregular, often they were subnormal. Vomiting was present in nine cases. Marked abdominal distension occurred twice, and pronounced nervous symptoms four times. The ages varied from one month to thirteen months.

Seven of these children died, five recovered, and two were improved. Four were admitted in June, six in July, and four in August. One autopsy could be made: a well-marked pseudomembranous colitis was found.

These cases were clinically severe types of diarrhœal disease occurring among ill-fed children of the poor. Our experience suggests that this type of dysentery bacillus can cause intense intestinal infections, at least in ill-conditioned infants.

Six infants showed the Flexner-Manila bacillus (Group C.).

The cases were grouped as follows: chronic gastro-intestinal disturbance, one case; mild acute dyspeptic diarrhœa, three cases; malnutrition followed by acute diarrhœa, one case; pseudo-membranous colitis, one case.

Mucus was present in the stools from all the children; blood, as flecks, was noted once only. Fever was not prominent. The children varied in age from two to twenty-one months. Two were admitted in June, two in July, and two in August. Four were discharged and two died. An autopsy in one fatal case showed pseudo-membranous ileo-colitis.

In thirteen infants more than one variety of the dysentery bacillus was isolated. The combinations were as follows[4]:

| | | |
|---|---|---|
| Groups | I and IV | 2 cases. |
| " | I and II | 3 " |
| " | I, II, and IV | 2 " |
| " | I, II, III, IV, and lactose "B" | 1 " |
| " | II and IV | 5 " |

These cases pursued as a whole a more severe clinical course than the cases of single infection. In twelve infants the diagnosis of ileo-colitis was made. The remaining infant was marantic and the subject of acute dyspeptic diarrhœa. Tuberculosis was present in two children: once as a miliary infection arising in a caseous bronchial gland, and once as pulmonary phthisis. In the latter case tuberculous ulcers were present in the intestines, besides which, both the Y and Manila type of dysentery bacilli were isolated. In eight children ulceration of the gut was diagnosed. On admission these cases were in fair or poor condition. Four were badly nourished and four were collapsed. They were not especially febrile. The temperatures varied from 97° to 104° F. All the movements contained mucus. In eleven cases pus in considerable quantities was present. The child with tuberculous ulcers passed mucous stools without pus or blood even upon microscopic examination. Pyelitis complicated one case. These infants varied in age from one to thirty months.

One child was admitted in June, five in July, and seven in

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[4] The groups arbitrarily indicated by Roman numerals are explained in the bacteriological section of this paper.

August. This seasonal admission is in conformity with the observation that the ulcerative disease tends to appear in the second half of the summer. Nine of the children died, three recovered, and one was improved on discharge. Seven autopsies were obtained. All showed ulcerative ileo-colitis.

In three instances the dysentery bacillus was isolated, but before its type could be determined with accuracy the cultures were lost. Hence these cases will not be described.

The next group comprises two children in which a lactose-fermenting dysentery-like organism was obtained. This organism was first described by Duval and Schorer,[5] and has now been included by Torrey[6] in the pseudo-dysentery group of bacilli. These children showed, one a mild, the other a severe dyspeptic diarrhœa. One child died. No autopsy was obtained.

Eleven cases of combined dysentery bacillus and streptococcus infection were studied. The bacilli consisted of the several types and occurred either singly or combined with one another.

Three of the children showed symptoms of toxic dyspeptic diarrhœa, seven of inflammatory ileo-colitis, and one of intestinal indigestion. One fatal case which came to autopsy showed pseudo-membranous colitis, pyelo-nephritis, and broncho-pneumonia.

The course of the disease in these infants, who ranged from six months or under to thirty months in age, differed materially in severity from infections of the dysentery bacillus alone. The movements always contained mucus, muco-pus was noted in six cases, and more or less blood in six cases. Drowsiness occurred frequently, one child had convulsions at the onset of the symptoms. Three of the children were admitted in June, seven in July, and one in August. Seven children died. Autopsies were made in six cases: two showed the lesions of dyspeptic diarrhœa, three of ulcerative ileo-colitis, and one of membranous colitis. Two children recovered and one improved.

In six cases streptococci only were encountered as the pathogenic bacterial species. In one child only was ileo-colitis noted.

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[5] Duval and Schorer, *Studies from the Rockefeller Institute*, 1904, ii, 42.

[6] Torrey, *The Journal of Experimental Medicine*, 1905, vii, 365.

The autopsy in this case showed ulcers in the lower ileum, and hemorrhages and thickening of the large intestine. The five remaining children were examples of malnutrition (two cases), relapsed dyspeptic diarrhœa (one case), acute dyspeptic diarrhœa, (one case), unrecorded (one case).

The stools were mucoid and rarely were pus and blood present. The age varied from three to seven months. Two children were admitted in June, three in July, and one in August. Two children died, one recovered, and three improved. These cases of streptococcal infection were less severe than the cases of combined streptococcal and dysentery bacillus infection.

In the next small group of five cases a pathogenic colon bacillus was isolated twice, a pathogenic bacillus pyocyaneus twice, and a non-pathogenic bacillus pyocyaneus once.

The clinical course of these cases was milder than in the other groups. They were classed with dyspeptic diarrhœa and indigestion. The stools were mucoid and pus and blood were not noted. The children varied in age from three to seventeen months. They had either recovered or improved on discharge.

A final group of ten cases is given in which no pathogenic organism or organism agglutinating with the patient's blood was found. In several of these repeated study of the stools could not be made, and no autopsy was obtained in the fatal cases. In nine cases the clinical diagnosis of toxic infection was made. Once ileo-colitis occurred. Four children were badly nourished; once the symptoms pointed to meningitis. The children varied in age from two to twenty-four months. Eight recovered and two died. The fatal cases were, a suspected meningitis, and an infant which was admitted in a collapsed condition. A detailed account of these cases seems unnecessary.

The study of this series of seventy-four cases of infantile diarrhœa has brought out the fact that forty-two, or 58.3 per cent. of the children yielded, in cultures made from the stools during life or the intestine after death, some variety of the dysentery bacillus, without admixture of streptococci. In eleven additional cases streptococci were associated with dysentery bacilli. Hence dysentery bacilli occurred in 73.1 per cent. of all the cases studied.

If one deduct the two cases in which the lactose-fermenting organism, probably not a true dysentery bacillus, was found, there remain fifty-one cases with dysentery bacilli or 70 per cent. of the whole.

A small number of the children yielded the streptococcus and a still smaller number Bacillus coli communis and Bacillus pyocyaneus as organisms which possibly were directly concerned with the production of the intestinal lesions and symptoms.

The manner of distribution of the several types of dysentery bacilli cannot be determined from this study. It would be unfair and quite without value to base any conclusions on so small a number of cases. But the cases show that the several types of dysentery bacilli now recognized occur most often singly; but that in a fair number of instances a mixture of types exists in the intestine. In considering the frequency of this mixture of types the results of previous studies of the dysentery bacilli as well as our own must be used carefully, for in order to recognize such mixtures the study of large numbers of individual colonies from a given case—a laborious task—is required. Such painstaking studies have been carried out rarely in the past.

If a comparison of the severity of symptoms and fatality be made with the character of dysenteric bacilli infection, it will be found that the most serious lesions and greatest fatality were present among the group of mixed dysenteric infections. Whether this observation is merely accidental, or whether combinations among the types of the bacilli produce more serious consequences than a single type acting alone, cannot be stated without much further study along this line. The observations which we record make this further study desirable.

The characters of the intestinal lesions found at autopsies were extremely various and without definite relation to the types of infecting dysentery bacilli. The lesions might be invisible to the naked eye. They might consist of congestion, or pseudomembrane or ulceration. It would appear that no distinction, except possibly as regards degree, can be drawn between the lesions produced by any of the given types of the dysentery bacillus.

The dysentry bacillus is confined chiefly to the intestinal tract and only rarely penetrates to the mesenteric glands and liver, in which situations it was found in two cases.

The identification of the several types of dysentery bacillus was made by the use of the usual cultural and morphological tests. In the cases studied by us stools containing microscopic or macroscopic pus were the most favorable for the isolation of Bacillus dysenteriæ, this organism being found in 93 per cent. of the cases containing pus. Subdivisions into types were made on the basis of fermentation of sugars and agglutination. The number of the bacilli yielded by the different cases was subject to great variations: in some instances almost pure cultures and in others only a few colonies, after patient search, were obtained.

It will not be necessary to describe in detail the results of the fermentation and agglutination studies. It should, however, be stated that the first were repeated at least three times in each instance. The period of observation was never less than twenty-five days. Two readings were usually made: at the end of five and of twenty-five days.

The cultures which we studied numbered two hundred. These consisted of 172 isolations made by us in the summer of 1904, ten cultures supplied by Dr. Bassett and obtained previously by him from infants, and eighteen cultures from other bacteriologists. The classification into four groups as proposed by Hiss[7] was employed in our studies.

Group I. Original Shiga bacillus group. Monosaccharids are fermented readily; maltose is split slowly and imperfectly. The quantity of acid produced from the first sugars coagulates the serum-water medium, but the amount produced from maltose causes reddening without coagulation. A bacillus agreeing with these group characters was found thirty-five times in fourteen cases, in four cases without admixture of the other types.

Group II. Ferments monosaccharids and mannite early and later ferments saccharose and malteso. This group is represented by the Y bacillus of Hiss. In our studies we found this bacillus ninety-one times in twenty-five cases, in fourteen of which it was the only type present.

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[7] Hiss, *The Journal of Medical Research*, 1904, xiii, 151.

Group III. This group differs very little in its fermentative properties from Group II, the only distinction being its more energetic action upon saccharose. However, in agglutinative properties it also differs from Group II. It is best represented by the Strong culture. We isolated this type of bacillus three times; it was not found independently of other types.

Group IV. The bacilli of this group ferment readily the monosaccharids, mannite, maltose, dextrin, and saccharose. They are represented by the Flexner-Manila bacillus. We isolated this organism forty-four times from sixteen cases. They occurred alone in six cases.

Besides this series of bacilli which are regarded as "true" dysentery bacilli we encountered several times the lactose-splitting bacillus first described by Duval and Schorer[8] in 1904 and later by Duval[9] who regarded it as a new type of dysentery bacillus. Torrey[10] has recently made a careful study of the lactose-fermenting, non-gas-producing dysentery-like bacilli obtained from the diarrhœal stools of children, which he subdivides into three subsidiary types as follows:

Bacillus "A" (Duval and Schorer) ferments the monosaccharids, mannite, and lactose, but not dextrin or saccharose. No member of this group was found in our cases.

Bacillus "B"(Duval and Schorer) ferments the monosaccharids, lactose, mannite, maltrose, and dextrin and slowly ferments lactose. Seven cultures of this organism were obtained from three cases. From two of the cases no "true" dysentery bacilli were obtained.

Bacillus "C" (Duval) ferments the monosaccharids, mannite, lactose, saccharose, malrose, and dextrin readily. No member of this group was obtained from our cases.

In our studies of agglutination we found that the patients' blood in 65.7 per cent. of the cases agglutinated the type of

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[8] Loc. cit.

[9] Duval: *Journal of the American Medical Association*, 1904, xliii, 382.

[10] Loc. cit.

Bacillus dysenteriæ isolated from the case, while the blood from the same patients agglutinated the Flexner-Manila type of Bacillus dysenteriæ in only 47.7 per cent. of the cases. Furthermore, the blood in 77.7 per cent. of cases yielding the Flexner-Manila organism agglutinated the same strain of Bacillus dysenteriæ, while in four instances no type of Bacillus dysenteriæ could be isolated though the blood from the cases agglutinated the Manila organism.

In the studies of agglutinations with representatives of the several types of dysentery bacilli and with Bacillus "B" of the lactose-fermenting group the method of Castelanni was employed and the readings were macroscopical. We possessed the following specimens of immune sera: (1) sent by Dr. Shiga from Japan; (2) Flexner-Manila goat serum sent by Dr. Flexner; (3) Y serum sent by Dr. Hiss; (4) rabbit sera prepared by us from "Shiga" and "Strong" and lactose "B" cultures.

We will not give in this place[11] the tables showing the details of our studies upon agglutination, but we will state the general conclusions which we have drawn from them.

The Shiga bacilli (Group I) absorb few agglutinines of the other groups of bacilli, while the Strong bacilli (Group III) partially absorb the agglutinines of the Y bacilli (Group II), and of the Shiga bacilli (Group I).

Absorption with bacilli belonging to Group II reduces the agglutinating value of all sera tested for Group IV and lactose "B," while the agglutinines for Group II in some sera are partially absorbed by Groups I, II, IV, and "B." Group II agglutinines, existing in its own serum, are unaffected by absorption with bacilli of other groups.

Absorption with bacilli of Group III reduces the value of some of the sera for Groups I, II, IV, and "B." The Group III agglutinines are absorbed from some sera by bacilli of Groups II, IV, and "B."

Bacilli of Group IV partially absorb from some sera the agglutinines of Groups II, III, and "B." The agglutinines of Group IV are absorbed in all instances by bacilli of Group II and sometimes by those of Groups III and "B."

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[11] A full presentation of the data upon which the clinical and bacteriological conclusions given here are based will appear in *The Johns Hopkins Hospital Reports.*

Lactose bacilli absorb the agglutinines of Groups II, III, and IV, while the agglutinines of "B" are absorbed by bacilli of Groups II, III, and IV.

The absorption of agglutinines by the different representatives of the groups of dysentery bacilli varies with the type of organism used in immunization. It is not improbable that a further variation will be found to depend upon the animal used for immunization. Our studies do not establish strong and definite specificity in agglutination among the several group-types, as the differences noted were usually those of degree only. Apparently the Flexner-Manila bacillus yields on immunization the greatest amount of common agglutinines since its serum is active against all the five group-types of bacilli studied by us.

# STUDIES UPON THE INFLUENCE OF TENSION IN THE DEGENERATION OF ELASTIC FIBRES OF BURIED AORTÆ.

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Plates XVII, XVIII, and XIX.

The experiments described in this paper were carried out with the object of studying the power of resistance possessed by elastic tissue to degenerative changes, the order of such changes as do occur when this resistance is overcome, and the effect of continuously applied tension upon these fibres.

Space does not permit of more than a brief review of the investigations already made by pathologists upon this tissue under various abnormal conditions. For more detail the reader is referred to a very concise paper by Jores, in Lubarsch and Ostertag's *Ergebnisse der allgemeinen Pathologie* (1902, Jahrgang VIII, p. 590).

*Disappearance of Elastic Fibres.*—Under various pathological conditions, organs, which show in health a large quantity of elastic tissue, exhibit a very marked decrease, if not total absence, of these fibres. The cause of this disappearance is much disputed. Du Mesnil de Rochemont considers that degeneration of elastic fibres is due to chemical action. He comes to this conclusion from observations made upon tuberculous material. He believes that the breaking down and disappearance of the elastic tissue is the result of free toxins which are the products of the bacillus of tuberculosis. In experimental pulmonary tuberculosis in animals produced by injecting cultures of the bacillus into the vascular system, Wechsberg noted a disappearance of the elastic fibres of the lung; this condition was recognizable within six hours and was very marked at the end of two days. He attributed it to the action of toxins of the micro-organism.

In sections of lupoid material, du Mesnil de Rochemont found a narrow marginal zone, about a line in width, which was free from these fibres. Here again he thinks that their absence is due to the action of toxins. Miller has observed the disappearance of this tissue in pulmonary gangrene and in syphilitic lungs within the areas of miliary gummata.

Obermüller, studying the changes in the vaginal wall in a case of prolapsus vaginæ, found an entire absence of elastic fibres in that part of the mucous membrane which was prolapsed and therefore exposed to mechanical irritation. This he thought was the result of fibrosis, secondary to the continual friction. The same author finds a similar absence of elastic fibres in those portions of the vaginal mucous membrane which have been subjected to prolonged pressure from constant wearing of a pessary. I can bear out Obermüller's observations concerning the disappearance of elastic fibres with prolapsus vaginæ. I have had the opportunity of examining sections from a case of prolapse of six years' duration. There was an almost entire absence of these fibres in the tunica propria and an extensive increase of white fibrous tissue. An obliterating endarteritis was also present.

Melnikow-Raswedenkow found, in testicular tuberculosis, a marked degeneration of the elastic fibres about the inflammatory zone of the tubercles, but in the necrotic centres he found the fibres intact. The explanation he advanced was, that the factor in the production of degeneration was the inflammatory cell infiltration and not the presence of toxin, since if the latter were the cause, the fibres would not remain in the necrotic tissue, which, one would suppose, would be surcharged with toxins. Schmaus finds that elastic fibres disappear very much more readily in the presence of granulation tissue than in true tubercles.

Jores agrees with the explanation offered by Melnikow-Raswedenkow. He quotes the work of his pupil; elastic tissue was exposed to inflammatory exudates and transudates at blood-heat in an incubator and was studied at various intervals, but in no case were changes found which at all resembled those occurring in inflammatory conditions during life though the tissue was in close contact with toxins which were present in the exudates and transudates.

In acute conditions a number of observers have found a

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irritation caused by these breaks. Katsurada experimented pinching the skin of animals, with forceps, and then examined e site of injury at different periods. He found that fragmentaon did not immediately follow the injury, but occurred when the owth of fibrous tissue, consequent to stimulation caused by e trauma, became excessive.

*Granular Degeneration.*—With one form of degeneration the lastic fibre is represented by a line of irregular granules which ke the elective elastic stains. Weiszmann, Neumann, and Manchot observed this form of degeneration in the early stages of arterio-fibrosis and aneurysm. Dmitrijeff found the same condition and obtained an elacin reaction in some of the granules. Miller has noticed this condition in the tubercles occurring with acute miliary tuberculosis. With syphilitic involvement of the vascular system, Abramow has observed extensive granular disintegration of the elastic fibres.

Jores has described a fatty degeneration of elastic fibres occurring in the absence of other signs of a degenerative process. In association with this condition he has noticed granulation of neighboring fibres and for this reason believes that both these changes are due to the same cause.

*Calcification.*—The elastic fibres may be impregnated by a lime salt; calcification occurs in cases of sclerosis involving the walls of blood-vessels or other tissues containing elastic fibres. Kockel has described incrustation of elastic fibres in calcified lung tissue. The deposit, he found, consisted of fine granules laid down along the fibres which were frequently broken. These granules stained well with resorcin-fuchsin. Von Davidsohn made a similar observation and likens the appearance presented by the fibre under these conditions to a chain of anthrax bacilli. Fragmentation in this tissue he considers analogous to fragmentatio myocardii. He does not believe that calcification begins in the fragmented fibres.

In some pathological conditions of the thyroid gland, Jores found calcareous degeneration of the elastic elements. The deposit was strictly limited to the elastic lamina. Matusewiez and Rona describe similar changes. The last-named writer found some of these granules occurring as inclusions in giant cells.

Upon removing the calcium salt from them he obtained a good elastic stain with orcëin and therefore was led to believe that the giant cells were responsible for the presence of calcium. In addition to the calcium salt, Rona found traces of iron. Gierke had shown, previous to Rona's observation, that iron is usually present with this form of degeneration.

*Methods.*—In order to study degenerative changes in normal elastic tissue an aorta obtained under aseptic conditions from the body of a healthy guinea-pig killed at the time required was buried within the tissues of another animal of the same species. The hair of this animal was removed over the site of operation by means of a depilatory powder.[1] Chloroform was administered to animals to be killed, but when recovery from the anæsthetic was desired it was found safer to employ a mixture of alcohol, chloroform, and ether (one, two, and six parts respectively).

The incision into the second pig extended through the skin, subcutaneous tissues, and the more superficial muscular tunic of the abdominal wall. With the handle of a scalpel a small pouch or pocket was formed by separating the muscular layers and into this pocket was placed the tissue to be buried. In order to prevent it from curling up, two anchor-ligatures of catgut were attached to the ends of the vessel; these ligatures were then led through the outer wall of the pouch, one at each end, and when drawn fairly taut were sealed to the skin. The wound was closed by catgut sutures and dressed with sterilized cotton and collodion.

In some instances (Groups B and C) in which it was desired to place the elastic elements under tension, the aortæ were distended under pressure with a sterile three per cent. solution of agar-agar in 0.85 per cent. sodium chloride, in other instances with sterile paraffin melting at 43° C.; the vessels were then doubly ligated at each end and excised.

The animals in which the collapsed vessels were buried were killed at the end of 3, 6, 12, 27, 33, 42, 54, 75, 106, 150, and 303 days, while those containing the distended aortæ, at the end of 10, 12, 15, 21, 27, 30, 35, and 50 days. After death the sections of abdominal wall containing the buried structures were removed, fixed and hardened in formalin (10 per cent.) solution and alcohol, and imbedded in paraffin.

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[1] A very good formula for this powder will be found in Crocker's work on Diseases of the Skin, in the section on Hypertrichosis.

The stains employed were hæmatoxylin and eosin, Van Gieson's fluid, resorcin-fuchsin, Weigert's elastic stain, orceïn, Unna-Taenzer's elastic stain, Wasserblau-safranin and three per cent. silver nitrate solution, and Schmorl's modification of v. Kossa's calcium reaction. Special tests for the presence of phosphates and chlorides were made.

### GROUP A.—AORTÆ BURIED IN UNDISTENDED CONDITION.

EXPERIMENT 1.—*Aorta after three days.* The vessel lies loosely imbedded in a quantity of granulation tissue, in the intermuscular space of the abdominal wall. The elements of the muscle and connective tissue of the buried aorta exhibit somewhat impaired reactions to the stains employed. The elastic structure of the vessel-wall stains well with the elective elastic tissue methods and exhibits no indication of a degenerative change. The lumen of the artery remains patent.

EXPERIMENT 2.—*Aorta after six days.* The buried vessel is firmly imbedded in recent granulation tissue. The lumen is occluded by a coagulum, in which commencing organization may be seen. There is a decided loss in quantity as well as in staining power of the muscle and connective tissues of the vessel but the elastic fibres are as yet apparently unchanged.

EXPERIMENT 3.—*Aorta after twelve days.* At this period the muscle and connective tissues have apparently been entirely absorbed, and the only remains of the buried vessel are the elastic framework. This structure is normal in appearance and to the elective stains shows a good reaction. The elastic fibrils, at the periphery of the vessel, exhibit a slight tendency to fragment, but this condition is strictly limited to fibrillar portions of the elastic structure.

EXPERIMENT 4.—*Aorta after twenty-seven days.* As in the previous specimen, the elastic tissue alone remains. A well-organized clot occupies the site of the occluded lumen. Though the elastic fibres generally take the specific stains well, yet there are small portions which indicate, by an impaired tingibility, an alteration of some kind. These areas occur chiefly among the peripheral and intimal fibres, the medial ones having so far escaped. In places also is seen a fairly well-marked fibrillation. No reaction is obtained upon using a special stain for degenerated elastin—the so-called elacin of Unna.

EXPERIMENT 5.—*Aorta after thirty-three days.* The aorta bears a close resemblance to the vessel buried twenty-seven days. In this specimen, however, the elacin reaction is obtainable, though it is not very marked. It appears to be confined to the internal elastic lamina. The newly forming connective tissue shows a tendency rather to compress than to invade the elastic structure. Fragmentation also occurs.

EXPERIMENT 6.—*Aorta after forty-two days.* An alteration of the staining powers of the elastic tissue in this specimen is very conspicuous. Both to resorcin-fuchsin and to acid orcëin these fibres exhibit a marked resistance, but when Wasserblau-safranin is employed they show extensive elacin degeneration. The internal elastic lamina shows several breaks, as do some of the other heavy fibres. Some of the fibres are fibrillating and the fibrils formed by this process of splitting-off are, in addition, undergoing granular disintegration. No reaction is obtained with Schmorl's calcium test. (Plate XVII, Fig. 5 and 6.)

Experiment 7.—*Aorta after fifty-four days.* There is a more marked loss of staining power with the elective stains than in the earlier specimens. Fibrillation, with its accompanying granular disintegration, and condensation of the elastic fibres continue. The elacin reaction is marked, but Schmorl's test for calcium yields only negative results.

Experiment 8.—*Aorta after seventy-five days.* Extensive changes in the elastic fibres are discernible. With both resorcin-fuchsin and acid orcëin there is a marked tendency to stain excessively and diffusely. The elacin reaction is present but not more marked than in the aorta after fifty-four days. With Schmorl's nitrate of silver test for calcium small dark areas are to be seen here and there in the course of some of the heavier fibres, including the lamina elastica interna. As the reaction is not marked there might be some doubt in stating that it is present at this date.

Experiment 9.—*Aorta after one hundred and six days.* There is marked hyper-tingibility of the elastic structure upon using the elective stains and there is the same heavy and diffuse reaction that is seen in the aorta after seventy-five days. With Wasserblau-safranin the reaction, though present, is less marked than in the previous specimen. Upon the application of Schmorl's test, however, there is seen an extensive calcareous deposition in the paths of the elastic fibres, especially of the lamina elastica interna. This deposition appears to be strictly limited to the fibres themselves—no deposit being seen except where granular disintegration has been marked. The sections cut with difficulty. (Plate XVIII, Fig. 7, 8, and 9.)

Experiment 10.—*Aorta after one hundred and fifty days.* The elastic structure is markedly condensed. The fibres show a hyper-tingibility similar to that found in the vessel buried one hundred and six days. The elacin reaction is absent, but Schmorl's test reveals a very complete impregnation of most of the elastic tissue by a calcium salt. Upon comparing one section of this aorta stained for calcium, with another stained with resorcin-fuchsin or with acid orcëin, the extent of this impregnation is better appreciated. Difficulty was encountered in cutting sections of this vessel also. (Plate XVIII, Fig. 10 and 11, Plate XIX, Fig. 12.)

Experiment 11.—*Aorta after three hundred and three days.* This specimen exhibits a large portion of the elastic structure of the buried vessel. The remains are, however, extensively broken and are calcified, but the extent of calcification is not so marked as in the specimen of one hundred and fifty days The elective elastic stains are taken excessively and diffusely. No reaction for elacin is obtained. (Plate XIX, Fig. 13, 14, and 15.)

## GROUP B.—AORTÆ DISTENDED WITH AGAR-AGAR AND BURIED.

Experiment 12.—*Aorta after twelve days.* The aorta is found well imbedded in granulation tissue. The distending medium, agar-agar, is broken into several parts by invading bands of newly forming tissue. The muscle and connective tissues have apparently been absorbed, the elastic structure of the vessel wall alone remaining. As a result of the tension placed upon the vessel during the period of experimentation, there is a marked diminution in the number of fibrils and smaller fibres present. A fibrillation of some of the larger fibres occurs, and fragmentation, though not marked, is present. The reaction to resorcin-fuchsin and to acid orcëin is apparently unaltered.

Experiment 13.—*Aorta after fifteen days; infected.* The effect of the continuous pressure is well shown in this specimen. Fragmentation, fibrillation and granular disintegration are present, the first not marked, the second and third more conspicuous. Very active cell invasion of the buried part is seen, and this no doubt accounts for the decrease in the peripheral fibrils. The tissue reacts well to the elective stains and no indications of elacin are found. In certain portions of the section the lamina elastica interna is found to be stripped from the subjacent structure and bulges into the lumen. This condition is caused by a collection of inflammatory cells beneath this membrane. In some places such as these the elastic fibres present an eroded appearance. In this specimen an additional factor has been infection. (Plate XVII, Fig. 1 and 2.)

Experiment 14.—*Aorta after twenty-one days.* A condition very similar to that described above is present. A decrease in the peripheral fibres and fibrillation are marked. The elective stains are well taken and there is no evidence of the presence of elacin. The fibres are not stretched to the same extent as in the other experiments. The distending medium is being rapidly absorbed, the lamina elastica interna shows numerous breaks and in places exhibits signs of erosion. Fragmentation though not marked occurs in other parts of the section.

Experiment 15—*Aorta after thirty days.* A marked decrease in the quantity of elastic tissue is at once noticed. Fragmentation is marked and the fibres are split and in some places are represented by granular masses. The internal elastic membrane is extensively broken and shows plainly indications of erosion. Only small portions of the agar-agar remain unabsorbed. The whole structure exhibits hyper-tingibility, but when treated with Wasserblau-safranin yields no indications of elacin. There is a marked tendency of the newly forming tissue to compress or condense rather than to invade the elastic structure. (Plate XVII, Fig. 3 and 4.)

## GROUP C.—AORTÆ DISTENDED WITH PARAFFIN AND BURIED.

Experiment 16.—*Aorta after ten days.* The vessel is imbedded in a quantity of new fibrous tissue. The muscle and connective tissues of the buried vessel cannot be identified. The elastic fibres are well shown, however, by the elective stains. There is a marked absence of fibrils and a slight breaking of the outermost fibres.

Experiment 17.—*Aorta after twenty-seven days.* The elastic fibres alone remain to represent the vessel buried in this experiment. These show marked fibrillation and granular degeneration, and the elastic structure as a whole, shows a decrease in volume. A reaction to the specific dyes is obtained, though there is no indication of elacin. In this experiment only a moderate amount of tension was exerted.

EXPERIMENT 18.—*Aorta after thirty-five days.* In this experiment a great deal of tension was exerted upon the vessel wall. Upon sectioning and staining, there is found to remain only a very small quantity of elastic tissue. The remains are fibrillated and granular and take the elective stains fairly well but exhibit no signs of elacin.

EXPERIMENT 19.—*Aorta after fifty days.* The degree of tension exerted about equalled that in the preceding experiment. There is absolutely no trace of elastic tissue to be found.

In studying the impregnation of elastic fibres by certain salts, it was found, as the result of the reactions given below, that calcium phosphate, principally, but also a small quantity of chloride were present. Klotz, by the use of Sudan III, demonstrated the formation of soaps preceding calcareous deposition. This test, however, was not applied to the tissues in my experiments.

For the demonstration of calcium, sections of the tissue were treated with a freshly made solution of hæmatoxylin (one fourth per cent.) in distilled water: while for phosphates and chlorides the reactions described by Professor A. B. Macallum and Schmorl's modification of von Kossa's test were used. In separation of chlorides from phosphates, the reagent used was that of Schmorl (three per cent. silver nitrate in distilled water plus one and a half per cent. nitric acid). Since silver phosphate is soluble in dilute nitric acid while the chloride is not, any subsequent darkening upon exposure of the sections to light indicated the presence of silver chloride.

From the study of the specimens described above, the following conclusions are, I believe warrantable:

1. Elastic tissue offers a very marked resistance to degenerative processes; this is especially noticeable when the changes which it undergoes are compared with those occurring in other tissues under similar conditions.

2. The power of resistance is lowered, apparently, when this tissue is placed under tension.

3. Degeneration of elastic fibres, when not influenced by ten-

sion, is indicated by the following changes which occur in the order given: (a) fibrillation or splitting of the fibres, (b) loss of tingibility, (c) fragmentation and granulation; then occurs either (d) absorption or (e) formation of elacin from elastin, followed by (f) a tendency to over and diffuse staining with the elective stains, and finally (g) impregnation of the elastic fibres with a calcium salt.

4. Under tension, elastic fibres may be absorbed without visible change except progressive thinning, or may fibrillate, undergo granular disintegration, and then be gradually absorbed.

5. Under tension and under the other conditions of these experiments, elastic fibres, apparently show no tendency to undergo impregnation by calcium salts.

6. The elective elastic stains are not true indicators of the condition of elastic fibres in certain stages of degeneration, unless used in conjunction with tests for elacin (Unna's method) and calcium (Schmorl's method).

In conclusion I wish to express my thanks to Professor J. J. MacKenzie at whose suggestion this work was undertaken and who has assisted me with his advice throughout its prosecution.

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## EXPLANATION OF PLATES.

### Plate XVII.

Fig. 1.—Low magnification of aorta buried fifteen days. Experiment 13. The elastic fibres are well stained. To the right, the lamina elastica interna is separated from the subjacent elastic fibres by a collection of inflammatory cells. To the left, fragmentation of the elastic fibres is observed. (Resorcin-fuchsin stain.)

Fig. 2.—High magnification of specimen shown in Fig. 1. In addition to the stripping off of the lamina elastica interna, the segmented condition of the fibres is well shown. (Resorcin-fuchsin stain.)

Fig. 3.—Low magnification of section of aorta buried thirty days. Experiment 15. The lower portion of this figure exhibits marked thinning of the elastic structure. The whole specimen shows extensive breaking of the elastic tissue, particularly noticeable to the left. (Resorcin-fuchsin stain.)

Fig. 4.—High magnification of specimen shown in Fig. 3. Segmentation of the fibres is shown. The elastic tissue stains well, but exhibits marked condensation. (Resorcin-fuchsin stain.)

Fig. 5.—Low magnification of an aorta buried forty-two days. Experiment 6. This photomicrograph shows well the impairment of tingibility of the elastic element—as seen in the lamina elastica interna and in some of the coarser fibres. Fragmentation is also shown. (Resorcin-fuchsin stain.)

Fig. 6.—High magnification of the specimen shown in Fig. 5. The inequality of staining of the elastic fibres in this specimen is very plainly brought out. (Resorcin-fuchsin stain.)

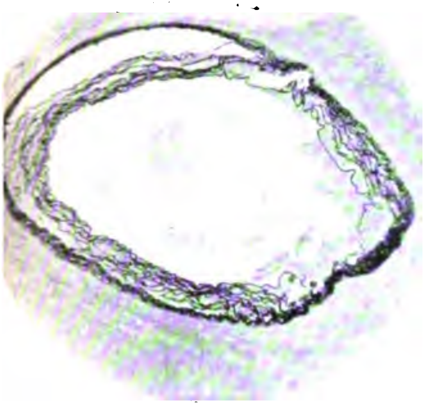
### Plate XVIII.

Fig. 7.—Low magnification of aorta buried one hundred and six days. Experiment 9. A marked hyper-tingibility of the remains of the elastic structure is present; the stain is taken deeply and diffusely. The elastic fibres show extensive segmentation. (Resorcin-fuchsin stain.)

Fig. 8.—Low magnification of the aorta shown in Fig. 7. (Schmorl's reaction for calcium.) Calcification of the fibres is very plainly demonstrated. The extent of the condition will be better appreciated if this figure be compared with the preceding one.

Fig. 9.—High magnification of same specimen as Fig. 7. (Resorcin-fuchsin stain.)

Fig. 10.—Low magnification of section of an aorta buried one hundred and fifty days. Experiment 10. This specimen exhibits hyper-tingibility which is very much more marked than that occurring in the aorta buried one hundred and six days. (Resorcin-fuchsin stain.)



Fg. 1.

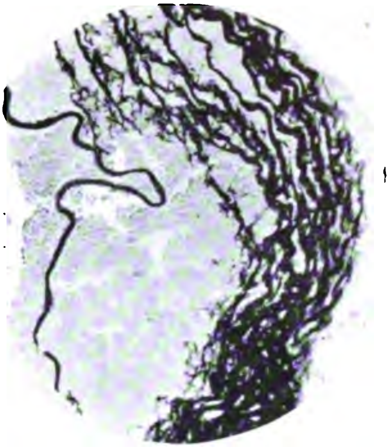


Fig. 2.

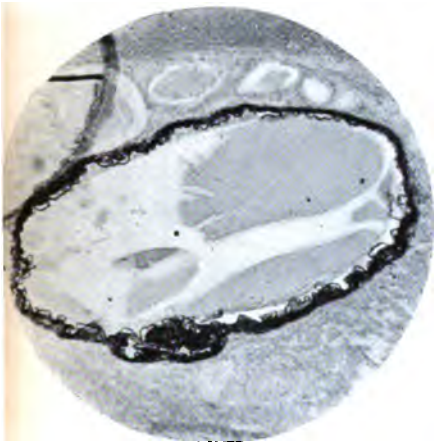


Fig. 3.

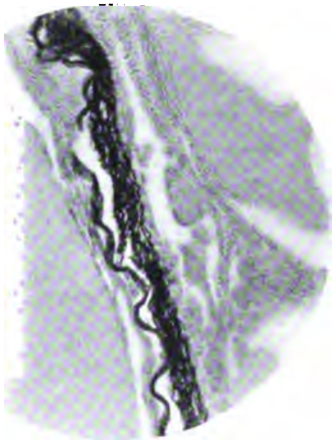


Fig. 4.

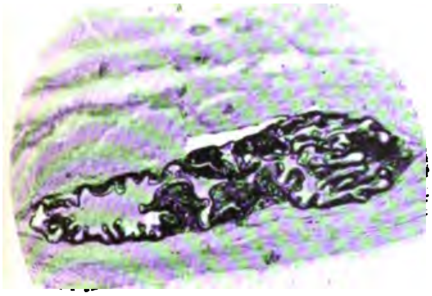


Fig. 5.

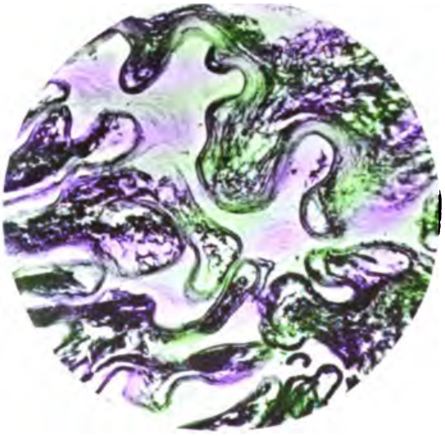


Fig. 6.

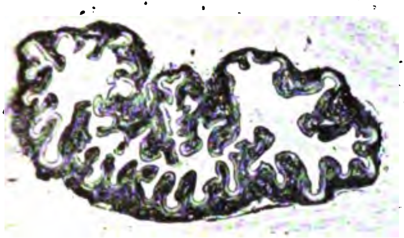


Fig. 7.

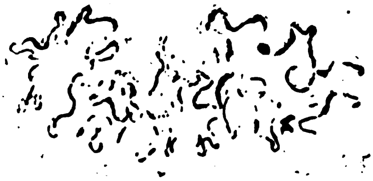


Fig. 8.

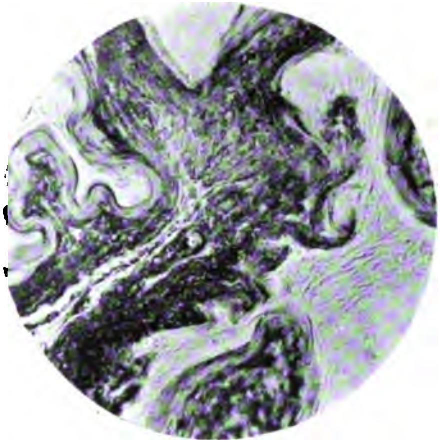


Fig. 9.

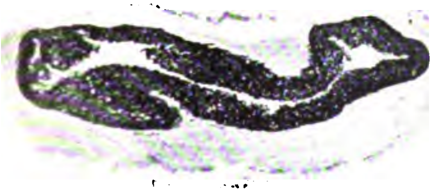


Fig. 10.

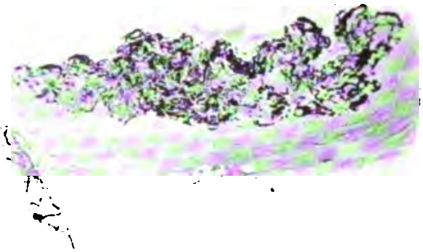


Fig. 11.

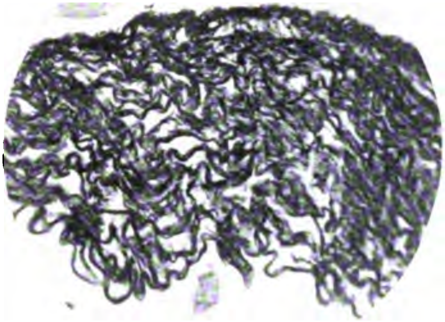


Fig. 12.

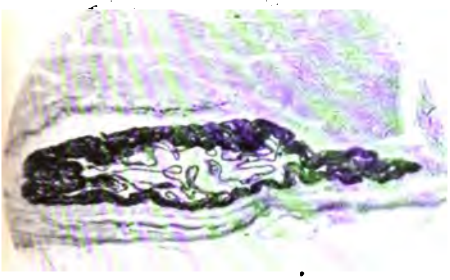


Fig. 13.

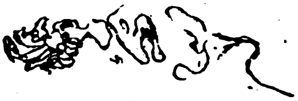


Fig. 14.

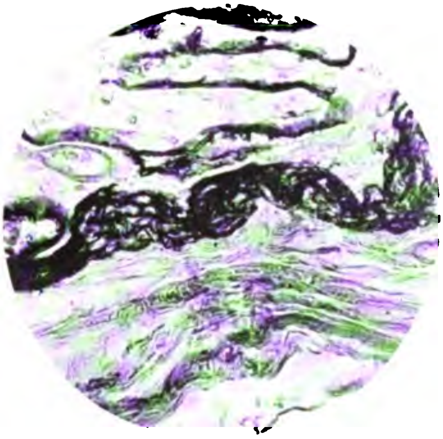


Fig. 15.

Fig 11.—Low magnification of the aorta shown in Fig. 10. (Schmorl's reaction for calcium.) Impregnation of the fibres with calcium is very marked and results in an almost complete cast of the original elastic framework.

## PLATE XIX.

Fig. 12.—High magnification of same specimen as Fig. 10. (Resorcin-fuchsin stain.)

Fig. 13.—Low magnification of an aorta buried three hundred and three days. Experiment 11. The fibres in this specimen exhibit a staining reaction which corresponds to that shown in Fig. 7. and 10. (Resorcin-fuchsin stain.)

Fig. 14.—Low magnification of same specimen as Fig. 13. (Schmorl's calcium reaction). The extent of calcification of the elastic fibres is shown. It is not nearly so extensive as that occurring in the aortas buried one hundred and six and one hundred and fifty days.

Fig. 15.—High magnification of same specimen as Fig. 13. (Resorcin-fuchsin stain.)

# EXPERIMENTAL MYOCARDITIS; A STUDY OF THE HISTOLOGICAL CHANGES FOLLOWING INTRAVENOUS INJECTIONS OF ADRENALIN.*

By RICHARD M. PEARCE, M. D.

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PLATE XX.

During a recent investigation[1] by Dr. Stanton and myself of the lesions occurring in the aorta of the rabbit as the result of repeated injections of adrenalin, the heart muscle, in the few instances in which it was examined, was seen not infrequently to be the seat of very definite degenerative lesions and occasionally also of proliferative changes. The association of such conditions with an experimental vascular lesion suggested the possibility of producing experimentally a chronic myocarditis analogous to that seen not infrequently in man in connection with grave arterial disease. As the material at hand was insufficient for a comprehensive study a second series of investigations was inaugurated. In these an attempt has been made to study the sequence of the changes and to determine to what extent they are due to the direct action of adrenalin and to what extent secondary to the arterial lesions. Few of the investigators who have produced vascular lesions with adrenalin have described the associated myocardial changes. Fischer[2] mentions the occurrence of necrotic and fibrous changes and, rarely, calcification. K. Ziegler,[3] in a series of eight animals, found changes very similar to those hereinafter detailed.

*Methods.*—These were practically the same as in the earlier experiments by Dr. Stanton and myself, except that the initial dose was placed at one-tenth cubic centimetre, and rapidly raised to five-tenths and occasionally higher. All injections

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* This investigation was conducted under a grant from the Rockefeller Institute for Medical Research. Read before the Johns Hopkins Medical Society, December 18, 1905.

were made into the ear vein, and usually on alternate days. As previous experiments had shown that ten to twelve injections of adrenalin were sufficient to produce well marked arterial lesions, the injections in this series with a few exceptions were thus limited and the animals were then allowed to remain without further treatment for periods varying from one to three weeks. Thus the animals which survived the treatment represent an average injection period of twenty-four days, and a total elapse of from twenty-seven to forty-five days, a sufficient time, as indicated by the few preliminary observations, for the development of proliferative lesions in the myocardium. The animals dying during the course of the injections furnished sufficient material for the study of early degenerative changes.

The histological methods were limited to the use of Scharlach R for frozen sections, and of hæmatoxylin and eosin and Mallory's connective tissue stain for material hardened in Zenker's fluid and imbedded in paraffin.

*Results.*—Thirty-six rabbits were injected. These may be divided into two groups: the first group including fourteen animals which died during the period of injection, and the second group, twenty-two animals, which received from seven to fifteen injections and were killed at various intervals thereafter. The first group represents animals receiving from one to five injections, of which none died later than the tenth day. All animals dying in this stage succumb apparently to circulatory and respiratory disturbance and present at autopsy acute dilatation of the heart, œdema and congestion of the lungs, and occasionally hæmorrhages from the serous and mucous membranes or in the interior of organs. No macroscopic lesions of the aorta are demonstrable. When death follows the injection within a few minutes the cardial dilatation, especially after the fourth or fifth injection, is extreme. Under such circumstances the heart loses much of its conical shape; the apex is more or less rounded and the over-distended auricles project laterally and greatly broaden the base. The left ventricle is very anæmic and its pale almost bloodless substance is in sharp contrast to the blue-black color of the thin walled auricles.

*Degenerative Changes.*—The heart muscle of two of three rabbits dying within a few minutes after a single injection shows a well marked œdema causing more or less separation of the muscle fibres: in the third animal the œdema is slight. In all three the muscle fibres show irregular swellings, and the striations are somewhat indistinct. In one, multiple fractures of more or less homogeneous fibres are frequently seen, but as these conditions could not be found in either of the other hearts, they were considered as probably artifacts.

But one animal died after two injections. This death occurred on the fourth day, thirty-six hours after the second injection. The œdema of the myocardium is so great as to cause wide separation of the muscle fibres in many areas. In some of these areas the fibres have lost their striations and are very granular. A few small areas of intense œdema take a diffuse blue stain similar to myxomatous tissue.

Three animals died after three injections. The notes on one of these follow:

Rabbit No. 25.—Male, weight 1670 grammes. The initial dose was one-tenth cubic centimetre of a one to one thousand solution of adrenalin injected into the ear vein. On the following day the dose was raised to two-tenths and after an interval of two days it was repeated. The animal was found dead seventeen hours after the last injection. At autopsy œdema and congestion of the lungs and extreme dilatation of the heart are present. Histologically the heart reveals focal areas of most intense œdema. These occur not only in the wall of the ventricle but also in the papillary muscles. Crossing these areas are strands of connective tissue in the midst of which fragments of heart muscle may occasionally be seen. Some of these fragments stain well while others are merely shadows of fibres in the midst of the finely granular coagulated serum. Some have ragged irregular ends pointing definitely to displacement by fracture. Elsewhere the fibres are widely separated by a serous fluid, are very granular, have swollen nuclei, stain lightly with eosin, and have but indistinct striations. On cross section many of the fibres show a clear zone about the nucleus, evidently a perinuclear œdema. Occasionally, bundles of fibres appear as if torn across, the space between being filled with serum. The branching of the fibres is brought out well by the œdema. Swollen hyaline fibres are not infrequent. The larger blood vessels are widely distended; the capillaries on the other hand are not prominent. No lesions are seen in the walls of the blood vessels.

In a second animal of this period which received three injections of one-tenth cubic centimetre the œdema was not so prominent, but on the other hand more extensive granular and hyaline degeneration of the fibres was present. After the fourth injection one animal died and after the fifth, six. These last represent the period of greatest mortality as well as the gravest degenerative lesions. All animals dying at this time showed extreme dilatation of the heart and histologically, in addition to the changes just described, a severe granular and hyaline transformation with more or less necrosis and disorganization. This condition is illustrated by the following protocol:

Rabbit No. 6.—Male, weight 1720 grammes. On September 26, 29, and 30 one-tenth cubic centimetre of adrenalin solution was injected into the ear vein; on October 2 and 3, two tenths. The animal died during the night of October 3. Upon post-mortem examination the heart was found to be dilated, the left ventricle pale and bloodless, and the lungs œdematous; the other organs were normal. Sections from three levels of the heart show general œdema and extreme disintegration of the muscle fibres. On both cross and longitudinal section the latter are seen to be irregularly swollen, granular, without striation, and not infrequently hyaline. The nuclei are swollen and frequently surrounded by a zone of œdema. In many fibres with extensive vacuolization of the protoplasm the nucleus does not stain. About such fibres small accumulations of mononuclear leucocytes are occasionally present and in some instances foci of epithelioid cells. In areas of extreme degeneration the outlines of the muscle fibres on cross section are indistinct and the picture is that of a uniformly granular mass with regularly placed lightly staining chromatic rings. These various changes affect the papillary muscles also. No changes in the vessels of the heart are demonstrable. Histological examination of the aorta is negative.

In other animals of this period the degenerative changes in the heart were of the same general character though varying more or less in degree. In two, a slight diffuse growth of connective tissue between the muscle fibres occurred.

Attempts to demonstrate fat in frozen sections of formalin hardened material treated with Scharlach R and counterstained by hæmatoxylin were uniformly negative. This method however revealed, in a few hearts, isolated fibres or small groups of fibres taking a diffuse yellow stain. These were more or less hyaline, and without nuclei; they however occurred in close relation to other similar fibres which did not thus react. This reaction has not as far as I am aware been previously described and its significance is not apparent.

The lesions in these hearts are due, manifestly, either to the direct toxic action of adrenalin upon the heart-muscle or to mechanical disturbances of the circulation caused by the effect of adrenalin on the vascular mechanism. They cannot be secondary to alterations in the walls of the large vessels, for in this series none of the animals exhibited macroscopic lesions of the aorta and in but three were degenerative changes evident microscopically.

*Proliferative Changes.*—Of the twenty-two animals survivng seven to fifteen injections, all but three showed degenerative changes in the myocardium, though seldom of the severe grade observed in the above descriptions. In twelve, more or less definite new connective tissue formation was evident. This new tissue was present, in some hearts, as focal accumulations of fine connective tissue about individual hyaline or granular fibres or about small groups of such fibres. In others a diffuse arrangement of new tissue was demonstrable in considerable areas of the myocardium and in two cases a very severe and diffuse fibrous myocarditis existed. The new tissue as a rule was most abundant about the blood vessels but had no relation to the sub-epicardial tissue or to the endocardium. It was very prominent in the papillary muscles. All parts of the ventricle suffered, though it any difference existed, the lesions were more severe at the apex. Focal accumulations of leucocytes were not prominent in the more chronic lesions, though occasionally present in the earlier conditions. When present these were usually of the lymphoid cell type. No suppuration or other evidence of infection was seen at any time; the lesion apparently was a pure connective tissue proliferation following degeneration of muscle fibres. The following protocol is that of the most advanced lesion observed:

Rabbit No. 22.—Male, weight 1685 grammes. During a period of eleven days seven injections of adrenalin varying in amount from one-tenth to eight-tenths cubic centimetre were given (total amount five and two-tenths cubic centimetres). The animal was killed by chloroform five days after the last injection.

*Autopsy.*—Weight 1650 grammes. A small amount of clear serous fluid is found in the peritoneal and pleural cavities. The heart is large and firm; the walls of the left ventricle are distinctly thickened; the auricles are dilated. The entire circumference of the aorta at its origin is the seat of closely massed, depressed, round or oval, calcified areas which form a complete ring about the vessel and extend irregularly into the sinuses of Valsalva and along the ascending portion of the aorta for a distance of one and two-tenths centimetres. The orifices of the coronary arteries, in the natural position of the aorta, are entirely obscured but by pressure on the heart substance their presence is indicated by the escape of minute points of blood. These lesions cause considerable deformity of the vessel. A few isolated plaques of similar appearance are scattered elsewhere in the aorta and a considerable number are grouped about the origin of the cœliac axis.

*Histology.*—Sections through the entire ventricle from base to apex show extreme degeneration of muscle fibres and a new formation of connective tissue diffusely arranged, for the most part, but with here and there focal areas in which the muscle fibres are entirely absent. The muscle fibres as a rule are swollen, vacuolated, and exceedingly granular, though some are atrophied. Fibres with irregular constriction and swelling and more or less hyaline transformation are not uncommon. The nuclei are distorted, stain poorly, and frequently are entirely absent. In some fibres the nucleus is contracted and surrounded by a clear zone; in other fibres the nucleus is greatly swollen. The connective tissue is more abundant in the mid and inner portions of the ventricle and but slight beneath the pericardium and in the papillary muscles. The focal areas are more numerous at the apex of the ventricle and towards the endocardial surface. The connective tissue is very loose and œdematous Between the elongated wavy vesicular nuclei is considerable intercellular substance practically free of lymphoid cells. While the arrangement of the new tissue is in general diffuse it is frequently more abundant about the blood vessels. In the focal areas the fibrillated character of the new tissue is very evident. The walls of the blood vessels show no change. Sections stained by Mallory's connective tissue method show everywhere between the muscle fibres a more or less well marked blue reticulum, while the focal areas take a deep stain relieved only by the deep red of fragments of hyaline fibres. The methods for demonstrating elastic tissue show a diffuse increase of fine elastic fibrils in the denser areas of connective tissue; they reveal no changes in the blood vessels.

*Control Observations.*—Although the literature treating of various experimental lesions of the myocardium of the rabbit contains no references to the occurrence of degenerative and proliferative changes in the hearts of apparently normal rabbits, it seemed advisable to determine definitely this point. As the sacrifice of a series of normal rabbits appeared undesirable the sections of heart muscle from a large number of animals injected with various organs and fluids of the dog during the past few years, in the course of several investigations of cytolysins, were utilized. A careful study of this material failed to reveal fibrous changes and showed few degenerative lesions. The latter were of slight degree and never of the type occurring after the administration of adrenalin.

*Mode of Action of Adrenalin.*—The association of a severe fibrous myocarditis with vascular lesions involving the orifices of the coronary arteries, as in the experiment given above in detail, suggests that the latter condition must have some causal relation to the myocardial changes. Indeed in the early part of the investigation it was thought that such a relation would eventually be demonstrated. Such, however, does not appear to be the case, for in one instance a myocarditis of equally severe type developed in the entire absence of lesions in the aorta. Moreover in six of the twelve animals exhibiting changes in the myocardium no lesions of the aorta were present, and in three of the remainder the lesions were but slight. The fibrous changes in the myocardium would appear therefore to be independent of the vascular lesions and not analogous to the myocarditis associated with arterial diseases in man.

Other possible explanations are based on a direct toxic action of adrenalin on the heart muscle, or an indirect action through the mechanical disturbances of the circulation which it causes. Although the physiological action of adrenalin on smooth muscle is well known we have no knowledge of its toxic action on the various types of muscle cells. Degenerative lesions in the parenchymatous cells and in muscle cells other than those of the heart and aorta are rarely seen, and it seems improbable that the toxic principle of adrenalin, if it exists, should be so selective as to affect only the muscle cells of the heart and the smooth muscle of the aorta and its larger branches. Indeed the limitation of degenerative lesions to the heart and larger blood vessels, and their practical absence in all other tissues, speak against any toxic action and point to an influence of a mechanical nature affecting these structures alone.

The action of adrenalin in constricting the arterioles with consequent increase in blood pressure is well known. This increase of blood pressure is alone not sufficient to explain the lesions, for if it were, we would expect the vascular changes due to adrenalin to be more general. But it is admissible to conceive of a temporary ischæmia of terminal vascular territories, due to the direct action of the drug upon the smooth muscle cells of the coronary arterioles, which lasts a sufficient length of time to cause some interference with the nutrition of the affected areas. From physiological experiments this period would appear to continue about ten to fifteen minutes. If to this we add the increased contractile effort of the heart necessary to overcome the greatly augmented intravascular tension, we have factors sufficient to explain the degenerative lesions of the early experiments. The heart muscle under conditions of poor nutrition is pushed to its extreme power and dilatation results with injury of single fibres or groups of fibres. This injury is due in part to nutritive and in part to mechanical disturbance. That the dilatation is extreme, that the muscle is anæmic, and that serious alterations of the fibres are associated with this condition after but a single injection of adrenalin has been repeatedly demonstrated during the course of this investigation.

Practically the same explanation was advanced by Dr. Stanton and myself to explain the necrosis in the lesions of the aorta due to adrenalin. K. Ziegler's explanation of the vascular lesions is also very similar. He points out that the inner and middle portions of the media of the aorta, the usual seat of necrosis, is the part of the vessel most scantily supplied by the vaso-vasorum and therefore most susceptible to necrosis at the time of vascular spasm and local anæmia.

*Comparison with Other Experimental Lesions of the Myocardium.*—The degenerative changes due to adrenalin do not conform in all respects to those caused by other agents used experimentally. The hyaline and granular changes in the muscle fibres, the alterations in striation, and the slight accumulations of lymphoid cells are similar to those found by various investigators (Comba,[4] Welch and Flexner,[5] and Mollard and Regaud[6]) who have studied the effect of diphtheria toxin on the heart muscle. On the other hand they differ in the absence of fatty changes, in the extreme œdema of the interstitial tissue, and in the disorganization of the fibres. In the same way also they differ from the lesions produced by Welch[7] in his experimental study of the effect of high temperature and from those produced by Ribbert[8] with Staphylococcus pyogenes aureus, by Charrin[9] with Bacillus pyocyaneus, by Tallquist[10] with Streptococcus pyogenes, by Björksten[11] with various bacteria or their toxins, and by Flexner[12] with ricin and abrin.

With the exception of the slight focal increase of connective tissue described by Charrin, Comba, and Mollard and Regaud, as a rare result of repeated injections of bacterial toxins, the only form of fibrous myocarditis mentioned in the literature of experimental lesions of the heart is that following ligation of the coronary arteries. The histological changes following such ligation have been described by Kolster, Porter, and Baumgarten[13]. The last, in his very detailed description, states that by the fourteenth day the new connective tissue had largely replaced the widespread areas of anæmic necrosis. This is of interest in connection with the lesions above described, in that the most marked connective tissue proliferation occurred in a rabbit killed sixteen days after the first injection.

*Relation to Lesions in Man.*—No definite comparisons can be made. The animals with acute degeneration of the myocardium and extreme dilatation of the heart which die suddenly after the fourth or fifth injection represent a condition apparently similar to that occasionally seen in man, where unusual strain causes sudden death due to an acute dilatation of a heart previously injured by some toxic agent or disturbance of nutrition. The later fibrous lesions have no analogy to the commoner chronic forms of myocarditis in man associated with arteriosclerosis or following known toxic conditions. That some of the more unusual forms of fibrous myocarditis in man which are difficult of explanation may be due to circulatory disturbances of the same general nature as those caused by adrenalin injections in the rabbit cannot be denied. It is to these only that the results of this experimental investigation appear to have any relation.

## EXPLANATION OF PLATE XX.

**Fig. 1.**—Fibrous myocarditis with more or less focal arrangement of the new tissue. Rabbit No. 22; see description in text.

**Fig. 2.**—Higher magnification of a different portion of the same section show-

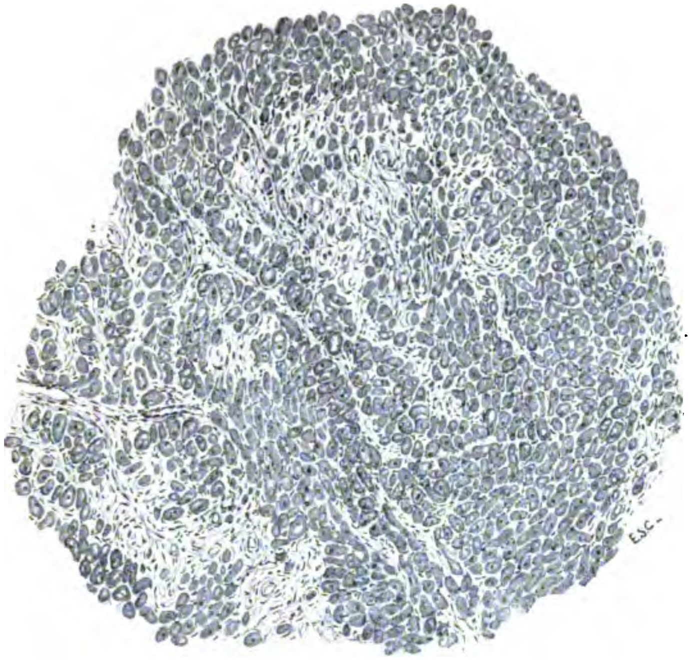
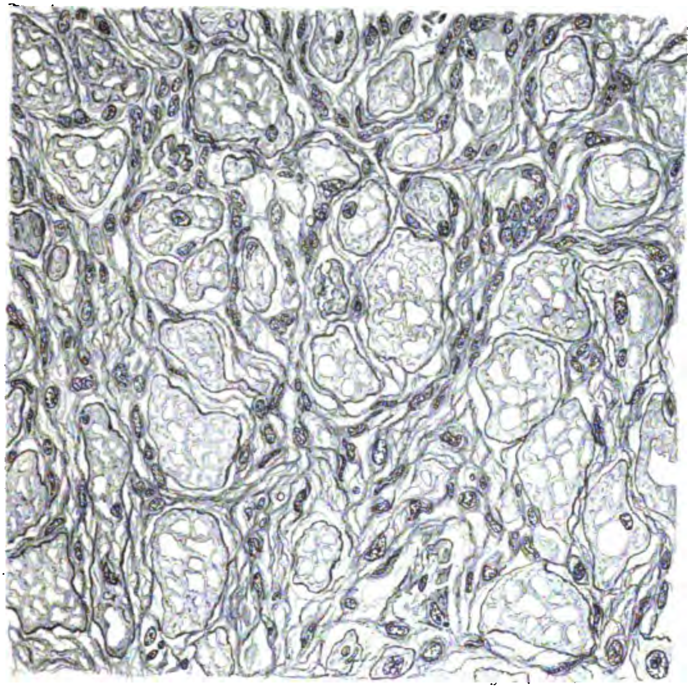


Fig. 1.



C.C.W.

Fig. 2.

ing the character of the muscle degeneration and also a moderately diffuse increase of connective tissue.

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# THE ENZYMES IN PHAGOCYTIC CELLS OF INFLAMMATORY EXUDATES.

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Those who have insisted upon the predominant importance of phagocytosis in the mechanism of natural and acquired immunity have not deserved Buchner's reproach that multiplied observation of this phenomenon can afford no explanation of its significance. Innumerable experiments have been divised to determine the conditions under which cells of various kinds ingest micro-örganisms and other substances and the fate of the ingested material. The conditions under which phagocytosis occurs are now fairly well understood, but less is known concerning intracellular digestion.

Metschnikoff[1] has shown that a close relation exists between phagocytosis in higher animals and the intracellular digestion of food by amœbæ, planarians, actinidæ, and other animals of relatively simple structure. Mouton[2] has studied the proteolytic enzyme which can be extracted with glycerine from the bodies of amœbæ and has found that it liquefies gelatin and digests feebly coagulated egg albumen; it acts both in a weakly acid and in a weakly alkaline medium. From the phagocytic cells which digest the food of actinidæ, or sea anemones, Krukenberg[3] and Mesnil[4] have obtained a similar enzyme which the latter has named actinodiastase. By analogy with the intracellular digestion of these invertebrates Metschnikoff has attempted to explain the process which occurs within phagocytic cells of vertebrates.

Common to all mammalian species are two types of cell capable of ingesting solid particles. One type is the polynuclear leucocyte

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[1] L'immunité dans des maladies infectieuses, Paris, 1901.

[2] *Annal. de l'Inst. Pasteur*, 1902, xvi, 457.

[3] *Untersuchungen a. d. physiol. Inst. d. Univ. Heidelberg*, 1878, ii, 273.

[4] *Annal. de l'Inst. Pasteur*, 1901, xv, 352.

(polymorphonuclear) with fine granulation, the neutrophile leucocyte of human blood, which leaves the blood vessels and forms the chief cellular element of most inflammatory exudates. A second type of phagocytic cell is larger than the polynuclear leucocyte and has usually a single large nucleus which is round or somewhat irregular in outline; its protoplasm does not contain granules exhibiting a specific reaction to dyes. The polynuclear leucocyte with specific granulation is readily recognizable either within or without the blood vessels and histological and experimental studies have shown that it is derived from cells with similar specific granulation which divide by karyokinetic division in the bone marrow. The characters of the large mononuclear phagocyte are less distinctive and the identity of cells of this type occurring in different situations under different conditions has been much disputed. Such large mononuclear phagocytes, which Metschnikoff has called macrophages to distinguish them from the smaller polynuclear cells or microphages, are always found in inflammatory exudates which have accumulated in serous cavities; during the late stages of the inflammatory process they are actively engaged ingesting and destroying red blood corpuscles, polynuclear leucocytes, and other cellular elements. The large mononuclear phagocytes of the spleen, lymphatic glands, and other organs which during typhoid fever ingest red blood corpuscles and lymphocytes are doubtless similar in character.

The two types of phagocytic cells react differently to different substances. Polynuclear leucocytes it is well known are attracted by almost all kinds of bacteria and are especially concerned in the inflammatory reaction which follows the invasion of these micro-organisms. When for example Staphylococcus pyogenes aureus, Bacillus pyocyaneus, or Bacillus coli is injected into the peritoneal cavity of the guinea-pig in quantity insufficient to cause its death they are quickly ingested by polynuclear leucocytes; a few bacteria are taken up by mononuclear phagocytes. When, however, cellular elements such as red blood corpuscles or spermatozoa are injected into the peritoneal cavity of this animal they are as Metschnikoff[5] has shown attacked and ingested by large mononuclear phagocytes or macrophages. Phagocytosis of certain parasites belonging to the animal kingdom, such as the malarial parasites and trypanosomes (Laveran and Mesnil[6]), is accomplished in great part at least by the same cells. The spirochæta of relapsing fever (Sawtschenko and Melkich[7]), and the similar parasite of geese (Metschnikoff[8]), organisms of which the zoölogical position is as yet undefined, suffer the same fate when injected into an insusceptible animal. It is noteworthy that certain bacteria, the bacillus of tuberculosis and the bacillus of leprosy (Iwanow[9]), are subject to phagocytosis by mononuclear cells, attracting in less degree polynuclear phagocytes.

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[5] *Annal. de l'Inst. Pasteur*, 1899, xiii, 737.

It has long been known that intracellular digestion by amœbæ and other protozoa occurs in the presence of acid. Granules of litmus ingested by some of these organisms assume a red color and alizarin-sulphate and other indicators of reaction show that the contents of the digestive vacuoles is acid. Similar observations have shown that the phagocytic cells which form the digestive cavity of certain low animal species such as sponges, planaria, and actinians, and by intracellular digestion prepare food for digestion, secrete acid into digestive vacuoles surrounding particles of food. Various substances ingested by such phagocytic cells assume in the presence of neutral red a brownish red color which according to Metschnikoff indicates the presence of acid. When phagocytic cells of the higher animals are allowed to ingest granules of litmus or tournesol-blue no change occurs; alizarin-sulphate is unaffected, for the reason, as Metschnikoff suggests, that it has a toxic action upon the phagocytic cell. When, however, a drop of exudate from a guinea-pig which has received in the peritoneal cavity nucleated red blood corpuscles from a bird is treated with a weak solution of neutral red, the partially digested corpuscles, or nuclei of corpuscles, which have been engulfed by macrophages assume the brownish red color which

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[6] *Annal. de l'Inst. Pasteur*, 1901, xv, 673.

[7] Ibid., 1901, xv, 497.

[8] L'immunité dans les maladies infectieuses, p. 169.

[9] *Annal. de l'Inst. Pasteur*, 1902, xvi, 705.

Metschnikoff attributes to a feebly acid reaction. J. Plato,[10] who first studied the effect of neutral red upon material ingested by phagocytes, found that bacteria are stained only after they are taken up by leucocytes and lose their color when the cell dies; he attributes the changes to oxidation and reduction of the dye, which with loss of oxygen becomes colorless. Himmel[11] has confirmed the observations of Plato; the reaction indicates, he believes, that intracellular digestion of bacteria occurs in the presence of a feebly acid reaction. Bacillus tuberculosis fails to exhibit this reaction even when ingested by the phagocytes of an insusceptible animal though other related bacteria which resist decolorization with acid assume a red color under the same conditions. Metschnikoff believes that intracellular digestion of bacteria usually occurs in the presence of a feebly acid reaction but may, as with the tubercle bacillus, occur in a weakly alkaline medium. The experiments which will be described do not support this opinion.

It is unnecessary to review the arguments with which Buchner, Metschnikoff, and others have maintained that the alexine or complement or cytase of the serum is contained in the leucocytes from which it is set free, according to Buchner by a process of secretion, or according to Metschnikoff by disintegration of cells (phagolysis), nor to consider the views of those who have opposed these theories. Buchner has suggested that the alexine is of the nature of an enzyme and not improbably is identical with the proteolytic ferment which is present within the leucocytes. Metschnikoff has claimed that the disintegration of red blood corpuscles which occurs within the macrophage is analogous to that which is produced by proteolytic enzymes within the digestive vacuoles of amœbæ and phagocytic cells of low invertebrate species. The enzyme or cytase which accomplishes intracellular digestion of bacteria is he believes identical with the body discovered in the serum by Nuttall and named by Buchner alexine but later more accurately defined by Ehrlich and designated complement. The observations which Metschnikoff has brought forward in support of his belief that the macrophages contain macrocytase or hæmolytic complement while the polynuclear leucocytes or microphages contain a second enzyme-like body, microcytase or bactericidal complement, will not be cited. Analogy with intracellular digestion among lower forms of animal life make it not improbable according to Metschnikoff that these bodies have the nature of proteolytic enzymes.

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[10] *Arch. für mikros. Anat.*, 1900, lvi, 868.

[11] *Annal. de l'Inst. Pasteur*, 1902, xvi, 663.

The occurrence of proteolytic enzymes in the cells of purulent exudates has been demonstrated by Fr. Müller, whose observations are recorded by Kossel.[12] A glycerine extract of purulent phthisical sputum was found to digest fibrin and coagulated albumen in a weakly alkaline medium. Other purulent sputa have the same power but purely mucous sputa do not exhibit it. Leber[13] has demonstrated the presence of proteolytic enzymes in purulent exudates and Achalme[14] has shown that they may liquefy gelatin and dissolve fibrin, coagulated egg albumen, and casein. By means of the Kjeldahl method, determining before and after digestion the amount of nitrogen contained in substances precipitable by tannic acid, Ascoli and Mareschi[15] have shown that the sediment from a sterile exudate obtained by the injection of aleuronat undergoes autolysis under toluol. Autolysis does not occur when the sediment has been subjected to a temperature of 60° C. during three hours.

Umber,[16] employing the method of Kjeldahl, found that the peritoneal fluid from two patients suffering with abdominal tumor underwent autolysis, but Schütz[17] found by the same method no measurable destruction of proteids though the exudates which he used contained in two instances a considerable number of leucocytes. Zak[18] studied pleural and peritoneal exudates from twelve patients. He found no autolysis in half of the cases and could demonstrate no relation between the abundance of cells and the degree of autolysis. A somewhat similar observation has been made by Hahn,[19] who has found that an artificially produced pleural exudate does not cause liquefaction of gelatin unless several days have elapsed since the injection of the inflammatory irritant.

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[12] *Zeit. für klin. Med.*, 1888, xiii, 149.

[13] Ueber die Enstehung der Entzündung, Leipzig, 1891.

[14] *Compt. rend. de la Soc. de Biol.*, 1899, li, 568.

[15] Eleventh Italian Congress for Internal Medicine, Pisa, October 27-31, 1901. Ref., *Maly's Jahresber.*, 1902, xxxii, 291.

[16] *Münchener med. Woch.*, 1902, xlix, 1169.

[17] *Zent. für inner. Med.*, 1902, xxiii, 1161.

[18] *Wiener klin. Woch.*, 1905, xviii, 376.

Experiments which I have described in a previous publication[20] have explained the discrepant observations just cited. The serum of the inflammatory exudate and of the blood inhibits the activity of the proteolytic enzymes contained in the leucocytes. When the leucocytes of the exudate are separated from the serum they undergo autolysis and are capable of digesting actively foreign proteid. In the late stages of inflammation produced by the injection of aleuronat into the pleural cavity of dogs there is some diminution of the anti-enzymotic action of the serum of the exudate.

*Methods.*—The methods employed have been described in the article to which reference has just been made. Exudates rich in leucocytes have been obtained by injecting a suspension of aleuronat in water containing starch (aleuronat, 5 grm.; starch, 1.5 grm., water, 100 c.c.) into the pleural cavities of dogs. Since the serum of the exudate inhibits the activity of the proteolytic enzymes contained in the leucocytes, it has been necessary to separate carefully cells from serum by repeated centrifugalization with normal salt solution (0.85 per cent.). In order to compare the activity of enzymes contained in leucocytes of different exudates the washed cells were measured in a graduated centrifugal tube after centrifugalization and suspended in a definite quantity of salt solution—equal to nine (1 : 10), nineteen (1 : 20), or twenty-nine (1 : 30) times the volume of the cells which were obtained. A measured quantity of the suspension of leucocytes was allowed to act upon heated blood serum, which being readily obtained formed a convenient means for testing proteolytic enzymes. Blood serum of the dog or of the ox mixed with an equal volume of salt solution does not coagulate, though it becomes opaque, when heated to 75° C. for one half hour, and may be accurately measured in a pipette. At this temperature the proteolytic enzymes of the serum described by Delezenne and Pozerski[21] and by Hedin,[22] as well as its anti-enzymotic action, are wholly destroyed.

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[19] Handbuch der pathogenen Mikroorganismen, herausgeg. von Kolle u. Wassermann, Jena, 1904, iv, 281.

[20] *Jour. of Exper. Med.*, 1905, vii, 316.

In all of the experiments to five cubic centimeters of heated serum (ten cubic centimeters of the diluted serum) were added in a small sterilized flask five cubic centimeters of the suspension of cells to be tested. Normal salt solution in sufficient quantity to bring the entire volume to twenty-five cubic centimeters was added, acid or alkali being substituted for salt solution in sufficient quantity to make any desired strength. After adding one cubic centimeter of toluol the flask was securely closed with a rubber stopper and kept at 37° C. in most instances during five days.

The same quantities of cells and of serum used for digestion were coagulated by heating to boiling and the nitrogen in uncoagulable form determined by the Kjeldahl method. The difference between the nitrogen of uncoagulable substances present in a flask kept at 37° C. and that of the control coagulated immediately represents the coagulable proteid converted by digestion into uncoagulable form. For the sake of convenience nitrogen determined by the Kjeldahl method will be represented by cubic centimeters of $\frac{1}{10}$ N sulphuric acid, since for comparison of results it is unnecessary to convert these figures into the corresponding quantities of nitrogen.

In the earlier experiments much difficulty was experienced in accurately coagulating the undigested proteid, complete coagulation being dependent upon a reaction which varies with the quantity of proteid. The method finally adopted was as follows: To each flask containing twenty-five cubic centimeters of fluid was added an equal volume of a sixteen per cent. solution of magnesium sulphate (20 grm. to 100 c.c. of water). After heating to boiling, acetic acid in sufficient quantity to react with litmus was added. After boiling, a weak solution of sodium hydroxide was added in such excess that on boiling again the coagulated proteids formed a flocculent coagulum which after cooling subsided and left the fluid perfectly clear. Since an excess of sodium hydroxide combines with magnesium sulphate to form insoluble magnesium hydroxide, the reaction of the solution is thus made approximately neutral.

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[21] *Compt. rend. de la Soc. de Biol.*, 1903, lv, 327, 690, 693.

[22] *Jour. of Physiol.*, 1904, xxx, 195.

In a publication previously cited I have shown that the enzymes contained in the leucocytes of an inflammatory exudate are capable of digesting proteid both in an alkaline and in an acid medium, though their activity is greater in the former. Hedin and Rowland[23] had shown that autolysis of parenchymatous organs, such as the spleen, kidneys, liver, heart, and lymphatic glands, occurs more actively in an acid than in an alkaline medium. From the spleen Hedin[24] has succeeded in isolating two enzymes, one of which acts with the aid of acid, the other in the presence of alkali. I have shown[25] that the bone marrow resembles the cells of an inflammatory exudate in its action on proteids, digesting far more actively in an alkaline than in an acid medium, and differs from other organs such as the liver, spleen, kidney, and lymphatic glands of which the proteolytic action is stronger in an acid medium. Since histologists with few exceptions are agreed that the polynuclear leucocytes with fine granulation are derived from the bone marrow, proteolysis produced in an alkaline medium by these cells may be referred to an enzyme formed in the bone marrow. The purpose of the following experiments has been to determine if leucocytes of the inflammatory exudate contain one enzyme which acts both in an acid and in an alkaline medium, or two enzymes one of which, like pepsin, acts in the presence of acid while the other, resembling trypsin, acts in the presence of alkali.

*Preliminary Experiments.*—Since the exudates obtained by the injection of aleuronat into the pleural cavities of the dog frequently, contain red blood corpuscles which are present in considerable number especially when the injection has been accompanied by injury to the lung, it was considered necessary to test the action of red blood corpuscles on heated serum and to exclude the possibility that they contain enzymes capable of causing proteolysis either in an acid or in an alkaline medium. The following experiment shows that the proteolytic action of these cells is insignificant.

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[23] *Zeit. für physiol. Chem.*, 1901, xxxii, 341, 531.

[24] *Jour. of Physiol.*, 1904, xxx, 155.

[25] *Jour. of Exper. Med.*, 1905, vii, 759.

Experiment I.—Mixtures containing 5 c.c. heated serum and 5 c.c. of a suspension of red blood corpuscles washed three times by centrifugalization and suspended in salt solution (in dilution of 1:20) were kept at 37° C. for five days.

| | |
|---|---|
| With 0.2% acetic acid | 3.05 c.c. |
| Without addition | 2.3 " |
| With 0.2% sodium carbonate | 2.45 " |
| Control | 2.15 " |

In the experiments which I have recorded in previous articles the ability of the enzymes of the exuded leucocytes to digest proteids in a medium made acid by acetic acid was tested. In order to exclude the possibility that proteolysis occurs only with acetic acid, other acids, namely hydrochloric and sulphuric, were used. By adding two and a half, five, or ten cubic centimeters of the $\frac{1}{10}$ N acids to each of a number of flasks containing five cubic centimeters of a suspension of leucocytes together with five cubic centimeters of heated serum and bringing the total volume of each flask to twenty-five cubic centimeters with normal salt solution, the strength of the acid present was made respectively $\frac{1}{100}$, $\frac{1}{50}$, and $\frac{1}{8}$ N. For the sake of comparison flasks containing sodium carbonate in the same strength were prepared.

Experiment II.—Leucocytes from an exudate removed from the pleural cavities of a dog five days after the injection of aleronat were suspended in nineteen times their volume (1:20) of salt solution. The following figures represent in cubic centimeters of $\frac{1}{10}$ N sulphuric acid the amounts of nitrogen in substances uncoagulable by heat present in 5 c.c. suspension of cells and 5 c.c. heated serum after digestion five days at 37° C. in the media which have been mentioned:

| | Acetic Acid | Sulphuric Acid | Hydrochloric Acid | Sodium Carbonate |
|---|---|---|---|---|
| $\frac{1}{25}$ N. | 21.15 c.c. | 5.25 c.c. | 4.4 c.c. | 20 35 c.c. |
| $\frac{1}{50}$ N. | 18.95 " | 18.2 " | 13.95 " | 28.05 " |
| $\frac{1}{100}$ N. | — | 20.7 " | 17.9 " | 29.05 " |

| | |
|---|---|
| Digestion without addition of acid or alkali | 24.55 c.c. |
| Control | 4.0 " |

It is noteworthy that the maximum digestion occurs in a slightly alkaline medium. When no addition has been made the normal alkalinity of the heated serum though diluted with four times its volume of fluid gives slight alkalinity to the medium. The efficiency of the three acids in favoring proteolysis varies considerably; sulphuric and hydrochloric acids in the same strength ($\frac{1}{25}$)N in which acetic acid favors digestion almost wholly prevent it, while in much weaker solution they cause active digestion. If, as it is not improbable, the maximum efficiency of hydrochloric acid is represented by a strength less than $\frac{1}{100}$ N the relative activity of the three acids follows the same order as their dissociation.

*Preservation of Proteolytic Enzymes of Leucocytes by Glycerine.*—In the experiments just described as well as in those recorded in previous publications the enzymotic action of cells has been tested immediately after removal from the animal. For certain experiments preservation of the enzymes was desirable and several methods of preserving them were attempted. Suspensions of cells in fifty per cent. glycerine preserved their ability to digest proteid in an acid as well as in an alkaline medium; proteolysis produced by the suspension in glycerine was only slightly less than that caused by the same cells diluted to the same extent with salt solution and used in the fresh state.

EXPERIMENT III.—Leucocytes from a dog killed five days after the injection of aleuronat into the pleural cavity were suspended in nine times their volume of salt solution (1:10). To a part of this suspension was added an equal volume of salt solution and the ability of the diluted suspension (1:20) to digest heated serum tested in the presence of 0.2% acetic acid, in 0.2% sodium carbonate, and with an unchanged reaction. To the remainder of the original suspension was added an equal volume of glycerine, making the dilution of the suspension 1:20. At the end of a week and again at the end of a month the action of 5 c.c. of the suspension was tested under conditions similar to those previously employed. In all instances digestion occurred during five days at 37° C.

| | Suspension of fresh cells | Suspension in glycerine after 1 wk. | Suspension in glycerine after 1 mo. |
|---|---|---|---|
| With 0.2% acetic acid | 20.9 c.c. | 17.5 c.c. | 18.8 c.c. |
| Neutral | 26.0 " | — | 26.55 " |
| With 0.2% sodium carbonate | 28.65 " | 26.95 " | 29.8 " |
| Control | 2.9 " | 4.0 " | 6.8 " |

At first sight the figures suggest that the activity of the enzyme has at the end of a month increased rather than diminished. It may be noted, however, that there is a corresponding increase in the figure representing the control, doubtless as the result of slow autolysis occurring in the glycerine extract kept at room temperature. When the figure representing the control is subtracted from that obtained at the end of digestion it is found that in an acid medium digestion is less active when the glycerine extract is used (13.5 c.c.) than with the fresh suspension (18 c.c.); activity of the enzyme has diminished slightly at the end of a month (12 c.c.). In an alkaline medium the glycerine extract is somewhat less active (22.95 c.c.) than the fresh suspension (25.75 c.c.) and has remained unchanged during a month (23 c.c.).

*Preservation of Proteolytic Enzyme of Leucocytes by Drying after Treatment with Alcohol and Ether.*—The attempt was made to determine if leucocytes dried and reduced to a powder retain their enzymotic action. The cells, obtained from an exudate by centrifugalization, after repeated washing with many times their volume of salt solution were treated with about fifteen times their volume of absolute alcohol; part of the alcohol was poured off after the cells had settled and replaced with fresh absolute alcohol. After standing about an hour the alcohol was removed by filtration and replaced with ether. After a short time the ether was removed by filtration and compression between pads of filter paper. The cells, allowed to dry in the air, were reduced in a mortar to a fine powder, which on account of the presence of a small amount of red blood corpuscles had a dull red color.

EXPERIMENT IV.—Leucocytes were obtained from a dog eight days after the injection of aleuronat into the left pleural cavity and dried by the method described. The dried powder (20 mgr.) was allowed to act upon heated serum (5 c.c.) in acid, neutral, and alkaline media for five days at 37° C.

| | |
|---|---|
| With 0.2% acetic acid | 7.55 c.c. |
| Without addition | 22.8 " |
| With 0.2% sodium carbonate | 18.8 " |
| Control | 5.8 " |

Experiment V.—Powdered leucocytes (20 mgr.) obtained from a dog two days after the injection of aleuronat acted on heated serum eight days at 37° C.

| | |
|---|---|
| With 0.2% acetic acid | 5.5 c.c. |
| Without addition | 19.05 " |
| With 0.2% sodium carbonate | 12.55 " |
| Control | 2.45 " |

Experiment VI.—The proteolytic activity of the dried enzyme from an exudate obtained twenty-four hours after the injection of aleuronat was tested four and a half months later with the following result:

| | |
|---|---|
| With 0.2% acetic acid | 6.3 c.c. |
| Without addition | 22.85 " |
| With 0.2% sodium carbonate | 21.1 " |
| Control | 5.35 " |

The powder prepared from leucocytes of a sterile exudate digests proteid actively in an almost neutral and in an alkaline medium but has almost completely lost the power of the fresh cells to digest in an acid medium. The most probable explanation of this fact is that the leucocytes of the exudate contain two ferments, one of which digests in a neutral or slightly alkaline medium, while the other acts in an acid medium; the former is uninjured during the process of preparing the dry powder, while the latter is destroyed. The enzyme which acts in an alkaline medium has preserved its activity unchanged more than four months in the dry condition.

For comparison with results to be described later an experiment showing the effect of various quantities of the enzyme upon a measured quantity of proteid is recorded.

Experiment VII.—Various quantities of the powder used in Experiment VI were allowed to act during five days at 37° C. upon 5 c.c. heated serum without addition of acid or alkali.

| | |
|---|---|
| With 10 mgr. powder | 19.05 c.c. |
| " 20 " " | 22.8 " |
| " 50 " " | 26.05 " |
| Control | 5.8 " |

The figures only very roughly indicate the velocity of the reaction produced by various quantities of ferment but show that a considerable increase in the amount of the latter causes a moderate increase in the amount of proteolysis at the end of a given time. E. Schütz,[26] studying by polarimetric estimation the amount of pepton formed from egg-white by the action of pepsin, reached the conclusion that the rapidity of digestion is proportional to the square root of the quantity of enzyme. Using Mett's tubes, in which a measured column of coagulated albumen is subjected to digestion, Borissow[27] and Samojloff[28] have obtained similar results. J. Schütz[29] has found that digestion may be shown to follow this rule by estimating by the Kjeldahl method the nitrogen of coagulable proteid converted into uncoagulable form. That the same rule is applicable to tryptic digestion has been maintained by Pawlow[39] and by Vernon.[31] For the present purpose the mathematical accuracy of these statements is not significant ; it is sufficient to point out that only a slight increase in the degree of proteolysis may be caused by large increase in the amount of enzyme.

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[26] *Zeit. für physiol. Chem.*, 1885, ix, 577.

*Effect of Temperature on the Proteolytic Enzymes of Leucocytes.*—Further evidence that the leucocytes of the inflammatory exudate contain two enzymes is furnished by the different effect of various temperatures upon proteolysis when tested in an acid and when tested in an alkaline medium. For this purpose suspensions of fresh cells were used; after five cubic centimeters of the suspension of cells in salt solution had been subjected to various temperatures for one half hour, five cubic centimeters of heated serum were added together with acid or alkali in quantity to bring the entire amount of fluid (25 c.c.) to a strength of 0.2 per cent.

EXPERIMENT VIII.—Leucocytes from an exudate removed from the pleural cavity of a dog twenty-three hours after the injection of aleuronat were suspended in nineteen times their volume of salt solution (1:20). The result of digestion caused by this suspension of cells heated to various temperatures was as follows:

| | In 0.2% acetic acid | In 0.2% sodium carbonate |
|---|---|---|
| Leucocytes unheated | 13.65 c.c. | 24.05 c..c |
| " heated at 50° C. | 13.5 " | 26.0 " |
| " " " 55° C. | 11.2 " | 27.4 " |
| " " " 60° C. | 8.7 " | 27.45 " |
| " " " 65° C. | 9.95 " | 25.65 " |
| " " " 100° C. | 4.0 " | 4.75 " |

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[27] Inaug. Diss., St. Petersburg, quoted by Pawlow.

[28] *Pflüger's Archiv*, 1901, lxxv, 86.

[29] *Zeit. für physiol. Chem.*, 1900, xxx, 1.

[30] The Work of the Digestive Glands (Trans.), London, 1902.

[31] *Jour. of Physiol.*, 1901, xxvi, 405.

**Experiment IX.**—Leucocytes obtained twenty-four hours after the injection of aleuronat were subjected to various temperatures as before.

| | In 0.2% acetic acid | In 0.2% sodium carbonate |
|---|---|---|
| Leucocytes unheated | 16.6 c.c. | 30.2 c.c. |
| " heated at 55° C. | 14. " | 32.85 " |
| " " " 60° C. | 12.45 " | — " |
| " " " 65° C. | 12.1 " | — " |
| " " " 70° C. | 8.65 " | 29.65 " |
| " " " 100° C. | 4.55 " | 5.2 " |

Temperatures between 55° and 70° C. diminish the enzymotic activity of the fresh leucocytes exhibited in an acid medium but do not materially diminish proteolysis in an alkaline medium. In an alkaline medium, indeed, the activity of proteolysis is slightly increased when the cells have been subjected to low temperatures. A somewhat similar observation has been made by Biernacki,[32] who found that heating trypsin in an alkaline solution in the presence of certain salts or of albumoses at 45° C. during from five to ten minutes increased its proteolytic activity so that digestion proceeded more rapidly than was usual.

Though the power to digest in an acid and in an alkaline medium is differently affected by temperatures below 70° C., proteolysis in both media is prevented by higher temperatures.

**Experiment X.**—Leucocytes removed from the pleural cavity of a dog five days after injection of aleuronat were suspended in nineteen times their volume of salt solution (1:20). Power to digest heated serum was tested in acid and in alkaline media after heating to temperatures between 70° and 100° C.

| | In 0.2% acetic acid | In 0.2% sodium carbonate |
|---|---|---|
| Leucocytes unheated | 16.35 c.c. | 25.0 c.c. |
| " heated at 70° C. | 4.75 " | 4.25 " |
| " " " 75° C. | 3.05 " | 3.6 " |
| " " " 80° C. | 3.65 " | 3.75 " |
| " " " 100° C. | 2.65 " | 3.65 " |

Although in this experiment the effect of heating at 70° C. is greater than in the two preceding experiments, it is evident that enzymotic activity of fresh cells is wholly destroyed at a temperature between 70° and 75° C. This statement applies only to the conditions of the experiment, since Biernacki has shown that reaction, and the presence of certain salts or of albumoses affect the ability of trypsin and pepsin to withstand various temperatures.

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[32] *Zeit. f. Biol.*, 1891, xxviii, 49.

*Changes in the Proteolytic Enzymes of Cells of Inflammatory Exudates Occurring during the Progress of Inflammation.*—When aleuronat is injected into the pleural cavity of the dog, as Buchner first showed, an active inflammatory reaction ensues and leucocytes together with a large quantity of serum accumulate in the cavity. At the end of from fifteen to twenty hours after injection of from five to ten cubic centimeters of a suspension of aleuronat there is a considerable quantity, in some cases more than one hundred cubic centimeters, of thin grayish-pink or red fluid containing usually not more than five per cent. of cells (measured after centrifugalization) among which red blood corpuscles are often numerous. Aleuronat is no longer free in the fluid but is firmly adherent to the pleural surfaces, accumulating along the anterior mediastinum just above the diaphragm. The lymphatic glands in the mediastinum, particularly those behind the upper part of the sternum, enlarge rapidly. After the injection of ten cubic centimeters of the suspension of aleuronat into each pleural cavity the latter usually contain at the end of from three to five days or even longer a considerable amount of fluid which is now much more turbid and richer in cells. Resolution of the exudate occurs, the aleuronat is absorbed, and except for the presence of fibrous adhesions the cavities return to their normal condition.

At the end of from sixteen to twenty-four hours after the injection polynuclear leucocytes with fine granulation form the greater number of the cells which are present. Differential counts show that from eighty to ninety per cent. of the leucocytes are of this type. The remainder of the cells are of the mononuclear type but few lymphocytes are present. The greater number of these mononuclear cells are somewhat larger than polynuclear leucocytes and have a large vesicular nucleus, which is usually somewhat irregular in outline, and fairly abundant protoplasm. They have the characters of the large mononuclear leucocytes of the blood. Since they are capable of active phagocytosis Metschnikoff has designated them macrophages; Kanthack and Hardy[33] and other English writers have called them hyaline cells; Marchand[34] describes them as leucocytoid cells.

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[33] *Jour. of Physiol.*, 1894, xvii, 81.

During the later stages of the inflammatory process polynuclear leucocytes with fine granulation are very numerous but their percentage is somewhat diminished since the large mononuclear cells have increased in number. Leucocytes with eosinophile or basophile granulation are present in insignificant number. At the end of three or five days large mononuclear cells form from twenty-five to thirty-five per cent. of the cells; the large proportion noted by Tarassévitch,[35] namely fifty to sixty per cent. two or three days after the injection of aleuronat into the pleural cavities of rabbits, was not found. The percentage counts of large mononuclear cells give little indication of the changes which they undergo, since as the exudate increases in age they increase in volume so that at the end of three or more days there are many cells with a diameter four or five times that of a polynuclear leucocyte. At this time very active phagocytosis is in progress and mononuclear cells with vacuolated protoplasm may contain red blood corpuscles or polynuclear leucocytes in various stages of disintegration; one large phagocyte may contain six or more of such cells. Mononuclear cells at this time are often clumped together to form masses which may contain from ten to fifty cells. The inflammatory reaction which follows the injection of aleuronat does not differ materially from that produced by the injection of various bacteria (described by Durham[36]) or by the injection of carmine, starch, or lycopodium powder (described by Marchand[37]) into guinea-pigs and other animals.

Since the cellular character of the exudate changes during the course of inflammation opportunity is afforded to study the relation between the proteolytic enzymes which are present and the types of phagocytic cells which have been described. For this purpose the power of a measured quantity of cells to digest a measured quantity of proteid has been tested in acid, in neutral, or in alkaline media. In the records which follow the amounts of digestion represented in cubic centimeters of $\frac{1}{10}$ N sulphuric acid have been obtained by subtracting the figure representing the control, coagulated immediately, from that representing the amount of nitrogen in substances uncoagulable by heat present after digestion for five days at 37° C. Differential counts of the leucocytes contained in the exudate have been made. Since cells with eosinophile or basophile granules have been rarely encountered, by polynuclear leucocytes are designated cells with fine granulation, corresponding to the neutrophile leucocytes of human blood. Mononuclear cells include not only the large mononuclear phagocytes but smaller cells resembling the lymphocytes of the blood; the latter form only a very small proportion, rarely so much as two or three per cent. of the number of cells.

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[34] *Verhandl. der deutsch. path. Gesellsch.*, 1898, i, 63.

[35] *Annal. de l'Inst. Pasteur*, 1902, xvi, 127.

[36] Loc. cit.

[37] Loc. cit.

It has been desirable to separate the experiments into several groups since in all instances the conditions of the experiment have not been the same.

SERIES A.—In the following experiments inflammatory exudates were obtained by injecting 10 c.c. of suspension of aleuronat into each pleural cavity of dogs which were killed at the end of one, two, four, and five days respectively. The washed leucocytes were suspended in nineteen times (1:20) their volume of salt solution. At the time these experiments were performed the method of comparison finally adopted was not contemplated so that the record is in some instances incomplete. The following figures represent the digestion of 5 c.c. heated serum produced by 5 c.c. of cell suspension kept at 37° C. during five days (save in Experiment XII in which digestion was stopped at the end of four days) in 0.2% sodium carbonate, in an approximately neutral medium, and in 0.2% acetic acid.

| Experiment | Age of exudate | Reaction of medium | | | Cells of exudate | |
|---|---|---|---|---|---|---|
| | | Alkaline | Neutral | Acid | Polynuclear | Mononuclear |
| XI | 1 day | 19.7 c.c. | | 9.3 c.c. | 84.8 % | 15.2 % |
| XII | 2 days | 0.3 " | | 7.3 " | 81.2 % | 18.8 % |
| XIII | 4 " | 0.6 " | 15.8 c.c. | 10.15 " | 73.8 % | 26.2 % |
| XIV | 5 " | 21.85 " | | 13.2 " | 78.2 % | 21.8 % |
| XV | 5 " | 24.05 " | 20.55 " | 17.15 " | 68.8 % | 31.2 % |

In Experiment XV (See Experiment II) digestion occurred in $\frac{1}{20}$ N sodium carbonate (approximately 0.2%) and in $\frac{1}{25}$ N acetic acid (approximately 0.24 %).

The ability of the fresh cells to digest in an alkaline medium shows considerable irregularity which has not been repeated in subsequent experiments; the almost total absence of digestion in Experiments XII and XIII has not been explained. In only two experiments was the power to digest in a neutral medium tested. With proteolysis in an acid medium there was an almost uniform increase as the time between the injection of the inflammatory irritant and the removal of the exudate increased.

A second series of two experiments is not comparable with those just described because the cell suspensions used were prepared by suspending cells in nine times their volume of salt solution (1:10).

SERIES B.—In the two experiments which follow the conditions are the same as those in Series A, save that washed leucocytes were suspended in nine times their volume of salt solution.

| Experiment | Age of exudate | Reaction of medium | | | Cells of exudate | |
|---|---|---|---|---|---|---|
| | | Alkaline | Neutral | Acid | Polynuclear | Mononuclear |
| XVI | 1 day | 28.0 c.c. | 16.95 c.c. | 12.5 c.c. | 84.0% | 16.0% |
| XVII | 5 days | 26.7 " | 18.55 " | 17.85 " | 73.6% | 26.4% |

Although the ability of the cells to digest in an alkaline or neutral medium has undergone little change, their efficiency in an acid medium has materially increased. At the same time the proportion of mononuclear cells has increased. It may be recalled on the one hand that the proportion of mononuclear cells does not accurately represent their relation to those with polymorphous nucleus since along with an increase in number the mononuclear phagocytes have undergone a great increase in volume, often having a diameter from three to five times as great as that of the polynuclear cells. On the other hand the amount of digestion during a given time does not increase in direct proportion to the amount of ferment contained in the cells but is more closely related to the square root of its quantity.

The exudates used in the preceding experiments were obtained by injecting a large amount of aleuronat, ten cubic centimeters of the suspension, into each pleural cavity. At the end of five days aleuronat is still present in great quantity adherent to the parietal pleura and embedded within the tissue of the anterior mediastinum. Doubtless as the result of the presence of the foreign body in large amount irritation is continued and the exudate fails to undergo resolution; at the end of five days there may be present in each pleural cavity 150 or 200 cubic centimeters of fluid. In the following experiments a smaller quantity of aleuronat, five cubic centimeters of the suspension, was injected into the right pleural cavity alone in the hope that long continued exudation of polynuclear leucocytes might be avoided. In an animal killed at the end of four days the right pleural cavity contained 75 cubic centimeters of fluid in which however the proportion of polynuclear leucocytes was still large.

Series C.—Cells obtained one and five days after the injection of 5 c.c. of aleuronat suspension into the right pleural cavity of dogs were suspended in nineteen times their volume of salt solution (1:20).

| Experiment | Age of exudate | Reaction of medium | | | Cells of exudate | |
|---|---|---|---|---|---|---|
| | | Alkaline | Neutral | Acid | Polynuclear | Mononuclear |
| XVIII | 1 day | 26.35 c.c. | 22.0 c.c. | 10.05 c.c. | 85.8% | 14.2% |
| XIX | 5 days | 25.75 " | 23.1 " | 18.0 " | 71.6% | 28.4% |

Series D.—In two experiments the conditions were identical with those just described save that washed leucocytes were suspended in twenty-nine times their volume of salt solution (1:30).

| Experiment | Age of exudate | Reaction of medium | | | Cells of exudate | |
|---|---|---|---|---|---|---|
| | | Alkaline | Neutral | Acid | Polynuclear | Mononuclear |
| XX | 1 day | 19.4 c.c. | 16.95 c.c. | 6.5 c.c. | 87.6% | 12.4% |
| XXI | 4 days | 17.2 " | 20.6 " | 12.9 " | 75.8% | 24.2% |

The results of the experiments of Series B, C, and D are uniform; with the progress of the inflammatory reaction there is an increase in the proportion of mononuclear cells and diminution in the percentage of polynuclear leucocytes, while corresponding with these changes there is a slight diminution of proteolysis in an alkaline medium and a well marked increase in an acid medium. On the one hand the polynuclear leucocytes have decreased from approximately eighty-five to seventy-five per cent. and on the other hand the mononuclear cells have almost doubled in number and at the same time have increased much in bulk. Evidence has already been produced to show that the cells of inflammatory exudates contain two enzymes characterized like pepsin and trypsin by their relation to acids and alkalis. The preceding experiments afford evidence that the polynuclear leucocytes contain that enzyme which is capable of active proteolysis both in an alkaline and in an almost neutral medium while the large mononuclear phagocytes produce that enzyme which digests proteid in an acid medium. It is not improbable that the latter enzyme produces slight proteolysis in a neutral medium since in Series B, C, and D there is with a marked increase of digestion in an acid medium a slight increase of digestion in a neutral medium.

Further evidence that the power to digest in an acid medium is dependent upon the mononuclear phagocytes is afforded by the following experiments in which were employed exudates produced by the injection of red blood corpuscles of the rabbit into the pleural cavity of the dog. Metschnikoff has shown that an exudate rich in mononuclear cells is obtained within twenty-four hours after the injection of red blood corpuscles of one species into an animal of another species. The large proportion of mononuclear cells noted by Gengou,[38] who found the exuded cells almost wholly of this type, was not obtained.

Series E.—Into each pleural cavity of two dogs were injected 5 c.c. of washed red blood corpuscles of the rabbit. In one animal, killed at the end of nineteen hours, the two cavities contained about 50 c.c. of deep red fluid from which 2.9 c.c. of cells were obtained by centrifugalization. In the two pleural cavities of the second animal, killed after three days, were 10 c.c. of thick pinkish-gray fluid from which were obtained 5.9 c.c. of cells. Suspensions of the leucocytes in both instances diluted in the proportion of 1:20 were allowed to act upon heated serum under the usual conditions.

| Experiment | Age of exudate | Reaction of medium | | | Cells of exudate | |
|---|---|---|---|---|---|---|
| | | Alkaline | Neutral | Acid | Polynuclear | Mononuclear |
| XXII | 19 hours | 23.9 c.c. | 20.65 c.c. | 14.3 c.c. | 63.6% | 36.4% |
| XXIII | 3 days | 21.65 " * | 19.5 " | 13.75 " | 63.2% | 36.8% |

*Digestion occurred in presence of 0.1% sodium carbonate.

The previously observed increase of proteolysis in an acid medium associated with an increase in the age of the exudate has not occurred when the proportion of mononuclear phagocytes has not undergone noteworthy change.

*Changes in the Proteolytic Activity of Cells of Lymphatic Glands*

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[38] *Annal. de l'Inst. Pasteur*, 1901, xv, 68.

*near the Site of Inflammation.*—The enlarged lymphatic glands which are situated in the neighborhood of the focus of inflammation afford further opportunity to study the enzymotic action of the large mononuclear phagocytes. Aleuronat injected into the pleural cavity of the dog rapidly accumulates, in part perhaps as the result of gravity, upon and within the anterior mediastinum, which at the end of from three to five days is converted into a thick firm mass of tissue within which aleuronat is still recognizable. Microscopical examination shows that masses of aleuronat are surrounded by polynuclear leucocytes which in the immediate neighborhood of the substance have not infrequently undergone necrosis. Mononuclear cells of the type previously described are numerous. In the anterior mediastinum above the level of the heart aleuronat is much less abundant, the tissue is moderately œdematous, and there are present at least a half dozen enlarged succulent lymphatic glands. Two larger than the others are situated immediately behind the upper part of the sternum and at the end of about three days after injection of the irritant measure one and a half centimeters in length.

Microscopical examination of these mediastinal lymphatic glands show that their sinuses are distended with fluid and contain even within twenty-four hours after the injection of aleuronat large mononuclear cells which do not differ from those found in the pleural exudate and are capable of ingesting red blood corpuscles and polynuclear leucocytes. These cells increase progressively in size and number and in some instances are so closely packed within the sinuses that the organ has the microscopic appearance of a mesentric lymphatic gland during typhoid fever. Not only within the sinuses but in the medullary cords as well are found such cells containing numerous partially disintegrated polynuclear leucocytes. In the sinuses and in the medullary cords free polynuclear leucocytes are numerous; the germinating centers of the follicles are enlarged and do not contain polynuclear leucocytes.

Since these lymphatic glands contain large mononuclear phagocytes massed together in large number it was thought not improbable that they would exhibit some change in their ability to produce proteolysis. Jacoby[39] has shown that the thymus gland undergoes autolysis' and Hedin and Rowland[40] have found that autolysis of lymphatic glands proceeds more rapidly in an acid than in an alkaline medium. In a previous publication I have shown that an emulsion of cells prepared from the mesenteric lymphatic glands of the dog is capable of digesting heated blood serum.

By scraping the gland with a knife and forcing the tissue through a small seize made with fine wire gauze, cells were completely separated from stroma. After washing the cells with salt solution they were measured in a graduated centrifugal tube and suspended in a definite quantity of salt solution. Since the normal mediastinal glands of the dog are too small to furnish cells in sufficient quantity for use it was necessary to compare the proteolytic activity of the inflamed mediastinal glands with glands distant from the site of inflammation. For this purpose the mesenteric glands were suitable. In the sinuses of these glands are often found a considerable number of large mononuclear cells resembling those found in the inflamed gland. In every instance the mesenteric glands were examined microscopically and compared with sections from one or more of the mediastinal· glands which were used. The sinuses of the latter were often packed with mononuclear phagocytes in diameter several times that of a polynuclear leucocyte while those of the mesenteric glands contained a moderate number of similar cells only slightly larger than polynuclear leucocytes and very rarely containing ingested material.

Experiment XXIV.—Into each pleural cavity were injected 10 c.c. of aleuronat. At the end of three days when the animal was killed there was no fluid in either cavity. The substernal lymphatic glands were large and succulent and together with several bronchial glands were used to prepare a suspension diluted with salt solution in the proportion of 1: 30. The proteolytic action of 5 c.c. of this suspension and of the same amount of a similarly prepared suspension from the mesenteric glands upon 5 c.c. of heated serum when kept two days at 37° C. is represented by the following figures:

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[39] *Beiträge z. chem. Physiol. u. Path.*, 1902, i, 147.

[40] *Zeit. für physiol. Chem.*, 1901, xxxii, 341, 531.

| | Mediastinal | Mesenteric |
|---|---|---|
| With 0.2% acetic acid | 11.45 c.c. | 9.9 c.c. |
| Without addition | 4.9 " | 5.2 " |
| With 0.2% sodium carbonate | 5.1 " | 5.05 " |

Control, 5.05 c.c.

Experiment XXV.—Suspensions, diluted 1:30, were prepared from the substernal and mesenteric lymphatic glands removed four days after the injection of 5 c.c. of aleuronat into the right pleural cavity of a dog.

| | Mediastinal | Mesenteric |
|---|---|---|
| With 0.2% acetic acid | 11.95 c.c. | 8.75 c.c. |
| With 0.2% sodium carbonate | 4.25 " | 3.8 " |

Control (approximately*), 3.3 c.c.

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* The quantity of cells obtainable from the lymphatic glands was insufficient for the preparation of a control flask. The amount of nitrogen in uncoagulable form contained in 5 c.c. of serum with 5 c.c. of a suspension of cells from the pleural exudate was determined. The amount of such nitrogen in this quantity of cells is insignificant.

The preceding experiments have shown that suspensions of cells from the inflamed mediastinal glands and from the relatively normal mesenteric glands cause proteolysis in an acid medium but are capable of causing little if any digestion in a neutral or alkaline medium. In both experiments greater proteolysis was caused by the inflamed than by the normal gland. The two experiments which follow confirm this observation.

Experiment XXVI.—The proteolytic activity of the cells from the mediastinal glands of a dog killed (A) nineteen hours after the injection of aleuronat was compared with that of cells from a dog allowed to live (B) five days after injection. From the latter animal a similarly prepared suspension (1:30) was obtained from the mesenteric lymphatic glands.

| | Mediastinal | Mesenteric |
|---|---|---|
| A—with 0.2% acetic acid | 7.6 c.c. | |
| B— " " " " | 12.2 " | 9.c.c. |

Control, A (approximately), 2.9 c.c.; B (approximately), 2.9 c.c.

In the following experiment an inflammatory reaction was caused by the injection of red blood corpuscles of the rabbit into the pleural cavities of the dog. The resulting histological changes in the mediastinal lymphatic glands do not differ materially from those produced by aleuronat. The sinuses contain in great number large mononuclear phagocytes actively engaged in ingesting polynuclear leucocytes and red blood corpuscles.

EXPERIMENT XXVII.—A suspension of cells obtained from the mediastinal lymphatic glands of the dog used in Experiment XXIII was diluted with salt solution in the proportion of 1:40 and compared with a similarly prepared suspension from the mesenteric glands. The result of digestion of heated serum during five days at 37° C. was as follows:

| | Mediastinal | Mesenteric |
|---|---|---|
| With 0.2% acetic acid | 7.85 c.c. | 5.85 c.c. |
| Control (approximately), 1.9 c.c. | | |

The foregoing experiments have shown that the proteolytic action of lymphatic glands containing large mononuclear phagocytes, which do not differ from those present in the exudate during the late stages of inflammation, is exhibited in the presence of acid and is almost wholly absent in a neutral or alkaline medium. In the dried powder prepared from washed leucocytes a proteolytic enzyme digesting in a neutral or alkaline medium was found almost wholly free from activity in the presence of acid. It has been possible therefore to obtain separately each of two enzymes demonstrable in the phagocytic cells which have a part in the inflammatory process.

As the length of time after injection of the inflammatory irritant increases mononuclear phagocytes increase in number and size and the exuded cells exhibit an increased ability to digest proteids in the presence of acid. In the neighboring lymphatic glands where cells of similar character accumulate there is an increased proteolytic activity in the presence of acid. The progressively increasing activity of similarly prepared suspensions from mediastinal glands at various intervals after the injection of aleuronat is indicated by the following figures obtained from Experiments XXV and XXVI (in which the conditions are the same) by subtracting the figure representing the control determination of nitrogen in uncoagulable form from that found at the end of digestion.

| | 1 day | 4 days | 5 days |
|---|---|---|---|
| With 0.2% acetic acid | 4.7 c.c. | 8.65 c.c. | 9.3 c.c. |

When it is recalled that the rapidity of digestion does not vary directly with the amount of ferment but is more closely related to the square root of this quantity the significance of these figures is increased: an increase in the number of mononuclear phagocytes is accompanied by an increase in the amount of that enzyme which digests proteid in the presence of acid.

*The Occurrence of Two Proteolytic Enzymes in the Cells of an Inflammatory Exudate and their Relation to Bone Marrow and Lymphoid Tissue.*—It has not been the purpose of the present study to determine the nature of the enzymes which have been found in the leucocytes of inflammatory exudates nor their relation to other somewhat similar bodies. The resistance of both enzymes to a temperature of 70° C. indicates that they are not identical with the alexine or complement, which is destroyed at approximately 56° C. Although the existence of two enzymes has been established, the one present in the polynuclear leucocytes, the other, in the large mononuclear phagocytes or macrophages, there is no evidence that they are identical with the bactericidal complement or microcytase and the hæmolytic complement or macrocytase which Metschnikoff thinks have their origin in these two types of cell. The experiments which have been described have not been concerned with the varieties of the complement nor with its relation to the leucocytes.

That enzyme of which the activity is limited to an acid medium resembles in this respect pepsin, while that which digests in an alkaline medium is more closely related to trypsin. On the one hand the ferment which limits its action to an acid medium is destroyed by means which are used to preserve pepsin, namely precipitation and drying with alcohol and ether, and fails to act in that concentration of hydrochloric acid which is most favorable to the action of pepsin, namely 0.2% (see Experiment II., $\frac{1}{25}$ N= 0.15%). On the other hand that enzyme which is contained in the polynuclear leucocytes and digests in an alkaline medium is apparently far less active than trypsin, obtained from the pancreas by similar methods.

It is not improbable that the enzyme of the mononuclear phagocytes is closely related to if not identical with the autolytic enzyme which is contained in various parenchymatous organs and acts more efficiently in an acid than in an alkaline medium. Repeated experiment has shown that the cells of an apparently normal lymphatic gland are capable not only of autolysis but of digesting foreign proteid (heated serum) in the presence of acid. Of especial significance, however, is the fact that the amount of enzyme increases when the gland contains an increased number of mononuclear cells capable of intracellular proteolysis.

Recent observers[41] who have studied the morphology and histogenesis of the various types of leucocyte found in the blood with few exceptions support the view long maintained by Ehrlich[42] that the lymphocytes and granular leucocytes differ in origin, one type having its origin in the lymphatic glands and other lymphatic structures, the other in the bone marrow. Concerning the nature of the large mononuclear leucocytes there has been greater divergence of opinion though most observers maintain with Ehrlich and Lazarus that they are closely related to the lymphocytes and have their origin in great part at least in lymphoid tissue. Marchand[43] for example believes that the large mononuclear phagocytes which accumulate in the peritoneal cavity after the injection of various irritant substances are derived from large mononuclear cells situated in the adventitia of the small blood vessels of the omentum and other structures. These cells he designates leucocytoid since they resemble similar cells found within the blood vessels and by division produce not only large phagocytes but he thinks smaller cells which do not differ from lymphocytes. Maximow[44] maintains that the large phagocytes which appear during the course of an inflammatory reaction are derived from lymphocytes which migrate from the blood vessels and has brought forward much experimental evidence in favor of this view. He believes that the adventitial cells of Marchand are of the same nature. Helly,[45] studying the exudates produced by the injection of bacteria into the peritoneal cavity, has reached conclusions similar to those of Maximow.

Corresponding with the two types of phagocyte, one the polynuclear leucocyte with fine granulation, the other the large non-granular cell derived from lymphoid tissue, there are two enzymes characterized by their ability to digest proteid, the one in a neutral or alkaline medium, the other in an acid medium. I have shown that the enzyme which is active in the presence of alkali occurs in the bone marrow and that the bone marrow, unlike the liver, spleen, kidney, and other organs, digests proteid more actively in an alkaline than in an acid medium. Since the polynuclear leucocytes with fine granulation—the neutrophile leucocytes of man—are formed in the bone marrow this enzyme doubtless has the same origin and may be designated *leuco-protease*. The name *lympho-protease* may be given to that enzyme which is contained in the large mononuclear phagocytes since these cells have their origin in lymphoid tissue.

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[41] See Sternberg, *Lubarsch-Ostertag's Ergebnisse der allg. Path.*, 1903, ix., Abt. ii, 360.

[42] Ehrlich and Lazarus, Normale und pathologische Histologie des Blutes, Nothnagel's spec. Path. u. Ther., 1900, Bd. viii, Tl. i.

[43] *Verhandl. der deutsch. path. Gesellsch.*, 1898, i, 63, and 1901, iv, 124.

[44] *Ziegler's Beiträge*, 1902, Suppl. Hft. v.

[45] *Ziegler's Beiträge*, 1905, xxxvii, 171.

# THE TOXICOLOGICAL CONSTITUTION OF AMANITA PHALLOIDES.

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It has been pointed out in previous papers[1] that the most deadly of all poisonous fungi, and the one responsible for the majority of deaths from fungus eating, is Amanita phalloides, the hæmolytic principle of which was discovered in 1891 by Kobert,[2] who gave it the name phallin. This hæmolysin, in virtue of its acting directly upon the red blood corpuscles without the intermediation of serum, its inactivation at 65° C., and its failure when inactivated to be reactivated by substances containing complement for serum hæmolysins, should be classed provisionally with the bacterial hæmolysins.

It has also been shown that extracts of Amanita phalloides are profoundly poisonous to animals, the latter showing lesions characteristic of bacterial intoxication in general, and that non-lethal doses are capable of causing an immunity in susceptible species. The serum from immunized animals is antihæmolytic and antitoxic in character, a minimal hæmolytic quantity for one cubic centimeter of a 5 per cent. blood suspension being neutralized by certain sera in a dilution of 1:10,000, and ten times a fatal dose for rabbits being neutralized by one cubic centimeter of the same sera. Such sera can be compared with the old and weaker normal antidiphtheritic sera where one cubic centimeter neutralized ten times a fatal dose of the diphtheria toxin for a guinea-pig of 250 grams. It is now necessary to consider in greater detail the lesions produced in animals by this poison, and to determine what relation these bear to the blood-laking properties of Amanita phalloides.

According to Kobert's original publications, there is but one poisonous substance in this fungus, phallin, and the changes seen in fatal intoxication in man and the lesions produced experimentally in animals by it are due entirely to blood destruction. Kobert regards phallin as a toxalbumen in virtue of its thermolability, and intravenous injections cause intravascular dissolution of the corpuscles. The specimen employed by him was of enormous hæmolytic strength, ox corpuscles being dissolved by the dried extract, in a dilution of 1:125,000.

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[1] Ford, *Medical News*, 1905, lxxxvii, 771. *The Jonrnal of Infectious Diseases*, 1906, iii., 191.

[2] Kobert, *St. Petersburger medicinische Wochenschrift*, 1891, xvi, 463; 471.

For the purposes of our present study, the lesions produced in experimental animals may be briefly stated to be the following[3]:

I. Subcutaneous gelatinous œdema;
II. Hæmorrhagic lymphatic glands;
III. Hæmoglobinuria;
IV. Hæmorrhages in serous membranes and parenchymatous organs;
V. Necrosis and fatty degeneration of heart muscle, liver, and kidney;
VI. Pigmentation of the spleen.

The pigmentation of the spleen and the hæmoglobinuria can be referred at once to the hæmolysis produced by extracts of Amanita phalloides, since these changes are characteristic of hæmolytic intoxications in general. Can we with equal confidence, however, consider the other changes, namely, the œdema, hæmorrhages, and necrosis, as due to hæmolysis?

If we inoculate a considerable series of animals, the lesions described are seen with great regularity. There is, however, a vast difference in the amount of œdema, the extent and distribution of hæmorrhages, and the degree of tissue degeneration. As we approach the lower limits of a fatal dose, the œdema at the site of inoculation becomes proportionately less, the hæmorrhages may be lacking altogether or at least the evidence of them at autopsy is wanting, and the extent of the tissue change, especially the fatty degeneration of the liver, becomes greater and greater. This variation in the character of the lesions suggests that the disturbances produced are greater than can be explained by blood destruction alone. If guinea-pigs, which are very susceptible to the toxic effects of this Amanita, be inoculated with minimal amounts of the poison they will frequently die in a short time and fail to present, at the site of inoculation, either œdema or hæmorrhage; and upon microscopical examination of the organs no pronounced lesions will be found. Nevertheless these animals develop much the same toxic symptoms as other animals which have received a larger fatal dose.

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[3] The lesions produced by Amanita phalloides will be more fully described in another paper.

If we estimate the amount of blood destruction produced by these small lethal doses of the Amanita, an unexpected fact is brought out: the degree of blood destruction is not proportional to the profundity of intoxication. A few examples will be given.

A guinea-pig of 240 grams weight succumbed quickly to a quantity of the Amanita which outside the body is capable of dissolving about one cubic centimeter of undiluted blood; and a rabbit of 870 grams weight died in forty-eight hours from a dose of the poison which dissolves only 2½ cubic centimeters of rabbit's blood. The loss of such small quantities of blood can be borne easily by animals of these species. In estimating the hæmolytic strength of doses of the extract capable of producing either rapid or slower fatal results, the same lack of proportion between the amount of blood destroyed and the severity of the lesions was apparent.

In endeavoring to explain the facts just stated several possibilities present themselves. The toxic effects produced by the Amanita may be secondary, in that the intravascular alteration of blood corpuscles may cause changes associated with thrombosis and infarction, to which the subsequent necrosis and tissue destruction are added; or the hæmolytic principle may be less specific than is supposed and exert an injurious effect upon other cells of the body; or finally, still other poisons in addition to the one discovered by Kobert may exist in the Amanita.

As regards the first of these considerations, it may be stated that Amanita phalloides does not agglutinate blood corpuscles *in vitro*, or, so far as can be concluded from a microscopic examination of the tissue, *intra vitam*. The tissue degeneration cannot, therefore, be explained by thrombi of agglutinated erythrocytes as some of the lesions produced by ricin and abrin have been accounted for. It is also no more easy to explain the lesions on the basis of simple destruction of a small number of red blood cells.

The second and third of these considerations can be tested experimentally. It has been pointed out that extracts of Amanita phalloides lose their hæmolytic activity entirely when heated to temperatures between 65° and 80° C., and no substance has been discovered capable of reactivating them. This inactivation may, therefore, for our present purpose be considered as destruction. Animals inoculated with extracts heated to various temperatures can be used to determine whether the entire toxicity disappears as the hæmolysins are rendered inactive. A series of rabbits and guinea-pigs was inoculated with different quantities of the Amanita extracts previously heated for one half hour to 60°, 65°, 70°, 80°, 90°, and 100° C. All the animals died after brief periods except those given the extract heated to 90° and 100° C. The extract heated to 60° C, caused, except for slighter subcutaneous œdema, lesions identical with those produced by the raw material. Hence a temperature of 60° C. fails to exert any special action upon the Amanita poison. The extract heated to 65°, to 70°, and to 80° C. is still toxic, killing animals almost as rapidly as the unheated material, but the pathological changes produced are greatly modified. Subcutaneous œdema and hæmoglobinuria are now absent; hæmorrhages are still present, but to a less degree, and the necrosis and fatty degeneration of the parenchymatous organs are diminished. Upon microscopic examination the pigmentation of the spleen is seen to be lacking, but all the other changes occur and are identical with those produced by the crude extract. With the loss of hæmolytic activity the extract has lost its power of producing subcutaneous œdema, hæmoglobinuria, and pigmentation of the spleen. The toxins are completely destroyed at a temperature between 90° and 100° C. The following protocols give the results of the heating experiments.

EXPERIMENT I. Rabbit, 1200 grams, inoculated February 18, 1905, with 1 c.c. of an extract of Amanita phalloides heated to 60° C. for ½ hour. Death Feb. 20. Autopsy: Subcutaneous œdema, hæmorrhages, and necrosis.

Experiment II. Rabbit, 1270 grams, inoculated Feb. 13, 1905, with the same, heated to 70° C. for ½ hour. Death Feb. 14. Autopsy: No exudate at site of inoculation. Hæmorrhages in lymphatic glands and adrenals. Liver congested; areas of hæmorrhage on its surface. Spleen dark and soft, bladder filled with normal looking urine. Hæmorrhagic areas in one lobe of the lung; a few minute hæmorrhages in the mucosa of the intestines. Cultures sterile.

Experiment III. Rabbit, 1445 grams, inoculated Feb. 13, 1905, with 1.2 c.c. of the same extract heated to 80° C. for ½ hour. Death Feb. 14. Autopsy: Slight congestion at site of inoculation, but no œdema. Lymphatic glands everywhere hæmorrhagic. Liver congested, but without definite areas of hæmorrhage. Spleen large, dark, and soft. Kidneys and adrenals show no definite hæmorrhages. Bladder filled with pale urine. Heart dilated; blood in heart and larger vessels clotted. Lungs, stomach, and intestines, negative. Cultures from the organs sterile.

Experiment IV. Rabbit, 1230 grams, inoculated Feb. 13, 1905, with 1 c.c. of the same extract heated to 90° C. for ½ hour. No symptoms; survived.

Experiment V. Rabbit, 1400 grams, inoculated Feb. 13, 1905, with 1.2 c.c of the same extract boiled for ½ hour. No effects.

Similar experiments which were carried out upon guinea-pigs gave the same definite results. The outcome of all these experiments shows that the extracts, when heated to 65° and 80° C., retain their toxicity, and cause death within forty-eight hours, but the lesions have entirely changed in character. In guinea-pigs a little œdema sometimes develops at the site of inoculation, but this is not of the same character as that produced by the unheated material. It probably merely indicates a great susceptibility of the blood-vessels to the poison, just as the corpuscles of the guinea-pig are so susceptible to the action of the Amanita that at times a very few corpuscles will be destroyed by the extract heated to 65° C. and even to 70° C.

Extracts of Amanita phalloides which have completely lost their hæmolytic activity still retain their toxicity. Hence after depriving these extracts of their phallin by heat there must still remain certain substances which are capable of causing rapid death, and of setting up those changes in the animal body associated with hemorrhage, necrosis, and fatty degeneration of the parenchymatous organs. To destroy completely the Amanita, temperatures of 90° to 100° C. are required. Hence I conclude that extracts of the fungus contain thermolabile and thermostable substances.

*Amanita Extracts Treated with Blood.*—Still another method for determining the toxic constitution of the Amanita is to treat the extracts with various cells of the body to ascertain whether they can thus be neutralized. If the extracts owe their toxicity primarily to a blood-laking poison, then the complete saturation of this poison by blood ought theoretically to give us a neutral mixture.

A number of combinations of corpuscles and the extracts were made for this purpose and the results were uniformly negative. Thus three cubic centimeters of an extract were mixed with twelve and a half cubic centimeters of washed rabbit corpuscles; hæmolysis occurred in about twenty minutes. This proportion of blood corpuscles and extract should theoretically give a neutral mixture, assuming the poison to be primarily a hæmolytic one. About eight cubic centimeters of this mixture, representing more than a fatal dose for certain animals, were given to a rabbit. Death took place after seven days and the post-mortem appearances were those of the Amanita intoxication.

If the extracts consisted chiefly of a blood poison whose activity is inhibited but not destroyed at 80° C., then the heated material should be capable of neutralization and be rendered non-toxic by saturation with blood corpuscles. Here also the experimental results were negative. Thus three cubic centimeters of an extract were combined with twelve and a half cubic centimeters of washed rabbit blood corpuscles, and eight cubic centimeters of this mixture were given to a rabbit. Such a mixture should theoretically be neutral. The inoculated animal, however, died at the end of twenty-four hours, and showed characteristic appearances of Amanita poisoning.

From these experiments it can be concluded that blood corpuscles are in themselves unable to neutralize Amanita extracts, and that the poisons contained in this fungus are not exclusively active against these cells.

*Extracts Treated with Brain Tissue.*—Many of the symptoms of Amanita intoxication point to a direct action of the fungus upon the nervous tissues. The experiments of Wassermann[4] upon the neutralization of tetanus toxin by emulsions of brain tissue and those of Flexner and Noguchi[5] upon the neurotoxic constituent of cobra venom have suggested another mode of analysis of the toxic constituents of this poison. If the chief poisons of the Amanita are neurotoxic, the blood-laking constituent being an ingredient of secondary importance, the combination of the Amanita extract with brain tissue should give a mixture acting still on the blood but without action on the nerve centres. Hence animals receiving this treated poison should recover, as the blood destruction is too small to cause, by itself, fatal intoxication. The results of this series of experiments are given in the following protocols:

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[4] Wassermann and Takaki, *Berliner klinische Wochenschrift*, 1898, xxv, 5.

EXPERIMENT I. The brain of a rabbit, excised aseptically, was macerated in salt solution and mixed with 5 c.c. of Amanita extract. After several hours' contact a guinea-pig of about 300 grams weight was inoculated with 1 c.c. of the supernatant fluid. Result: death in 18 hours; usual autopsy findings.

EXPERIMENT II. The brain of a guinea-pig, excised aseptically, was macerated in salt solution and mixed with 2 c.c. of a similar extract. The mixture was kept three hours at room temperature and then injected subcutaneously into a guinea-pig of 650 grams. Death in 18 hours.

EXPERIMENT III. A whole brain of a guinea-pig, excised aseptically, was macerated in salt solution and mixed with 4 c.c. of the extract. Two and one-half c.c. of this emulsion were given to a guinea-pig of 400 grams weight Death in 24 hours.

From these experiments, it may be concluded that brain tissue has not the power to remove or neutralize the poisonous principles of Amanita phalloides.

Since, however, the experiments with heat indicate that the thermolabile substance is definitely hæmolytic, it may be that the thermostable substance is neurotoxic in character, and hence the effect of the inoculation of the crude material depends upon the presence of these two poisons. It was desirable, therefore, to destroy the hæmolytic principle by heat and to extract the heated poison with brain tissue in order to determine whether the thermostable substance is really a neurotoxin. The toxicity could not be removed in this manner as the following experiment shows:

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[5] Flexner and Noguchi, *University of Pennsylvania Medical Bulletin*, 1902–03, xv, 345.

An extract of the Amanita was heated to 80° C. to destroy completely the hæmolysin, and 2 c.c. were mixed with the brain of a guinea-pig, which had been removed aseptically and emulsified in salt solution. The entire mixture was injected into a 400-gram guinea-pig, which died in two weeks and exhibited changes characteristic of Amanita poisoning.

I therefore conclude that the fungus does not contain a neurotoxin capable of being neutralized by brain tissue.

*Effect of Artificial Digestion upon Extracts of the Amanita.*—The fungus having been shown to contain two distinct principles—one thermolabile and the other more heat resistant—it is of interest to ascertain whether they show any differences to the action of ferments. To test this question the crude extracts, before and after heating, were digested for a number of days with pepsin and pancreatin and their hæmolytic strength and general toxicity ascertained. Table I shows that the unheated extract employed caused complete hæmolysis of 1 c.c. of a 5 per cent. suspension of rabbit corpuscles in quantities at and above 0.003 c.c., while 0.001 c.c. produced a trace of hæmolysis.

TABLE I.

| Crude extract of Amanita. | 5% rabbit blood. | Hæmolysis. |
|---|---|---|
| 1.0 c.c. | 1 c.c. | Complete |
| 0.1 " | " " | " |
| 0.05 " | " " | " |
| 0.01 " | " " | " |
| 0.005 " | " " | " |
| 0.003 " | " " | " |
| 0.001 " | " " | Trace |
| Control 1 c.c. Na Cl 1% | " " | None |

This experiment was repeated separately with pepsin and pancreatin. In each case the crude extract was digested for eight days with pepsin and with pancreatin, and in both the hæmolysin was entirely destroyed, since when mixed in equal quantities—one cubic centimeter of the extract and one cubic centimeter of the blood suspension—no hæmolysis occurred.

The influence of the ferments on the thermostable poison was either wholly negative, or, at best, very slight.

Experiment I. Guinea-pig weighing 670 grams. Injected subcutaneously 2 c.c. extract heated to 65° C. for ½ hour and digested with pepsin for 7 days. Death in 24 hours. The autopsy showed the characteristic lesions of he heated extract including extensive hæmorrhage at the site of inoculation.

Experiment II. Guinea-pig, 440 grams, inoculated with 1 c.c. of the extract heated to 65° C. and digested with pancreatin. Dead in 24 hours. Autopsy revealed usual changes.

Experiment III. Guinea-pig, 447 grams, inoculated subcutaneously with 1 c.c. extract heated to 80° C. for ½ hour and digested with pepsin for 7 days. Dead in 24 hours. Autopsy: Hæmorrhage at site of inoculation, and dilated heart.

Experiment IV. Guinea-pig, 395 grams, inoculated with extract heated to 80° C. and then digested with pancreatin for 7 days. Death followed inoculation within a very short time, possibly less than 1 hour. The material given to this animal, owing to its evaporation, represented a considerable multiple of the fatal dose.

These experiments show that the toxic substance in the Amanita which resists heat also resists the action of pepsin and pancreatin. That the crude extract is, moreover, still toxic after artificial digestion for two weeks, is shown by the fact that a dose of two cubic centimeters killed a guinea-pig weighing 420 grams in four days.

*Constitution of the Amanita.*—From these experiments dealing with the action of heat and of ferments upon extracts of the Amanita, and from the attempt to neutralize this substance with blood and nerve tissue, and, finally, from a consideration of the post-mortem changes, we obtain a different idea of the constitution of the fungus from the one held by Kobert. While the hæmolytic ingredient, the phallin of Kobert, is an important constituent of the fungus and exerts a very powerful action upon all erythrocytes, it is by no means the only substance present which exerts a toxic effect on animals. In addition to phallin, which is thermolabile and susceptible to artificial digestion, there is present a powerful thermostable substance resistant to the action of ferments. To the hæmolysin are to be ascribed the subcutaneous œdema, hæmoglobinuria, and pigmentation of the spleen produced in artificial intoxication; while to the action of the thermostable substance, which is non-hæmolytic, are due the hæmorrhages, the necrosis, and fatty degeneration in various organs. Whether the hæmolytic substance can produce necrosis, fatty degeneration, and hæmorrhage cannot at the present time be stated definitely, since the different ingredients of Amanita have been separated by chemical means, the thermolabile substance having been obtained free from the thermostable body, only within the past few weeks. It is hardly conceivable, however, that a substance acting so energetically upon blood corpuscles should not also exert a necrotizing action upon other cells of the body. The thermostable body is the most important constituent of Amanita and the one causing the most profound disturbance of the organs and tissues.

It has been pointed out in a previous paper that by the immunization of animals it is possible to obtain antibodies to extracts of Amanita phalloides which are both antihæmolytic and antitoxic in character. In view of the observations outlined in this paper the question may be asked whether this antiserum is a single body capable of neutralizing both the hæmolytic and the toxic action of the Amanita, or whether it is a combination of antibodies, each acting upon the especial substance to which the animal is immunized. To put the question in another way, it may be asked whether the particular chemical groups under whose influence the antibodies for the Amanita are produced, are identical in the thermolabile and the thermostable substances, or are quite different. If, according to Ehrlich's ideas, it be granted that every toxin is composed of two chemical complexes, one the haptophoric group uniting with susceptible cells, the other the toxophoric group causing the injury of these cells, the haptophoric group alone taking part in the processes leading to artificial immunity and the production of antibodies; then in the case of Amanita the toxophoric group of the hæmolytic or thermolabile constituent must be quite distinct from the toxophoric group of the thermostable constituent; since these groups attack different cells of the body, the one acting primarily upon the erythrocytes, the other primarily upon the cells of the parenchymatous organs. Two possibilities are, however, open in considering the haptophoric groups of these two constituents of the fungus. On the one hand, the haptophoric groups may have the same structure, in which case but one antibody should be found in the antiserum, the antithermolabile substance being identical with the antithermostable substance. On the other hand, the haptophoric groups may be different and thus the antiserum contain two distinct antibodies, one neutralizing the hæmolysin, the other neutralizing the resistant body.

If the hæmolysin of Amanita is destroyed by heat, and an antibody be made from the residue, and if the thermolabile and the thermostable bodies are identical in haptophoric structure, the antibody produced should be antihæmolytic and at the same time antitoxic. If, on the other hand, the haptophoric groups be different, the antibody produced for the thermostable body should be merely antitoxic and have no antihæmolytic power. ✓ Such an experiment should also settle definitely the question whether there are two separate toxic substances in Amanita phalloides, in contradistinction to the notion that the action of heat and of digestion is merely to alter the arrangement of molecules in a toxic complex consisting of a single substance, in which occurs one haptophoric group attached to at least two toxophoric groups presenting differences of resistance.

Jacoby,[6] who has investigated the toxic and agglutinating action of ricin, arrived at the conclusion that this poison contains an agglutinin and a toxin, each of which possesses separate and distinct haptophoric groups. At Ehrlich's suggestion, however, Jacoby immunized a goat to ricin from which all traces of agglutinin had been removed by combination with blood corpuscles, and as the resulting antiserum was anti-agglutinating as well as antitoxic, Jacoby was led to believe that the ricin-toxin and the ricin-agglutinin possessed identical haptophoric groups.

A series of animals was immunized to extracts of Amanita heated respectively to 65° C. and to 80° C. for one half hour. It proved to be somewhat less difficult to immunize the animals to the heated than to the unheated extracts, since the local œdema and necrosis at the site of inoculation were by this means largely avoided. The immunization was pushed to the point where the animals withstood the inoculation of several multiples of the fatal dose of the heated material, after which the blood was withdrawn and tested for its antihæmolytic and its antitoxic properties.

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[6] Jacoby, *Hofmeister's Beiträge*, 1902, ii, 535.

A rabbit of 1970 grams was given on successive occasions an extract of Amanita which had been previously heated to 65° C. for one-half hour. The acutely fatal dose was 1.2 cubic centimeters.

| Date. | Dosage. |
|---|---|
| May 5 | 0.3 c.c. |
| " 12 | 0.6 " |
| " 19 | 0.9 " |
| " 26 | 1.2 " |
| June 10 | 1.4 " |
| " 19 | 1.75 " |
| " 28 | 2.00 " |

Bled June 29, 20 c.c.

This serum was tested for its antihæmolytic and antitoxic effects.

TABLE II.

Action of Immune Serum on Hæmolysis.

| Extract. | 5 % rabbit blood. | Serum. | Hæmolysis. |
|---|---|---|---|
| .005 c.c. | + 1 c.c. | + 0 | = Complete |
| .005 " | + 1 " | + 0.1 c.c. Normal | = " |
| .005 " | + 1 " | + 0.05 " Immune | = " |
| .005 " | + 1 " | + 0.1 " " | = " |

The immune serum made from the heated extract does not prevent hæmolysis. The next experiments were made in order to test the antitoxic properties of the serum.

Control: 1 c.c. of an extract heated to 65° C. for ½ hour kills a rabbit of 1500 grams in a short time.

Experiment I. Rabbit, 1050 grams, inoculated July 1 with 1 c.c. of the extract heated to 65° C. for ½ hour + 1.5 c.c. of the immune serum. Animal unaffected.

Experiment II. Rabbit, 1150 grams, inoculated July 1 with 1 c.c. of the extract heated to 65° C. for ½ hour + 1.5 c.c. of the immune serum. Animal unaffected.

The immune serum, although completely devoid of antihæmolytic action, therefore possesses definite antitoxic properties.

A second rabbit was immunized to the Amanita extract heated to 80° C. for half an hour, and its serum was tested both for antihæmolytic and for antitoxic properties. The dose of the extract which quickly caused death was 1.2 cubic centimeters.

A rabbit weighing 1860 grams was immunized as follows:

| Date. | | Dosage. |
|---|---|---|
| May | 5 | 0.3 c.c. |
| " | 12 | 0.6 " |
| " | 19 | 0.9 " |
| " | 26 | 1.2 " |
| June | 6 | 1.4 " |
| " | 16 | 1.7 " |
| " | 26 | 2.0 " |

July 5, 15 c.c. blood withdrawn.

The serum of this animal was wholly devoid of antihæmolytic properties, being without effect in a 1 : 10 dilution. Its antitoxic action was marked.

Experiment I. Rabbit, weight 1500 grams, inoculated July 6 with 2 c.c of the extract heated to 80° C. ½ hour + 2 c.c. antiserum. Recovered.

Experiment II. Rabbit, weight 1500 grams, inoculated July 6 with 2 c.c. of the extract heated to 80° C. for ½ hour + 1 c.c. antiserum. Died.

Experiment III. Rabbit, weight 1600 grams, inoculated July 6 with 2 c.c. of the extract heated to 80° C. for ½ hour + 0.5 c.c. antiserum. Recovered.

The antiserum pôssesses, therefore, definite antitoxic properties, two cubic centimeters completely neutralizing an excess of the toxin representing about five times the lethal dose for a rabbit of this weight. Quantities less than two cubic centimeters failed to protect regularly. The antiserum is also effective for guinea-pigs.

Control: I c.c. of the heated extract kills a guinea-pig in 24 hours.

Experiment I. Guinea-pig inoculated July 6 with 1 c.c. of the extract heated to 80° C. for ½ hour + 1 c.c. antiserum. Recovered.

Experiment II. Guinea-pig inoculated July 6 with 1 c.c. of the extract heated to 80° C. for ½ hour + ½ c.c. antiserum. Recovered.

Experiment III. Guinea-pig inoculated July 6 with 1 c.c. of the extract heated to 80° C. for ½ hour + ¼ c.c. antiserum. Recovered.

We thus see that the antiserum produced by the immunization of animals to the thermostable substance contained in the extract of Amanita phalloides heated to 65° and to 80° C. neutralizes the toxic action of this substance only, having no power to neutralize the thermolabile or hæmolytic principle. The two substances must, therefore, possess separate haptophoric and toxophoric groups. They do not, therefore, consist of a complex molecule composed of a single haptophore group united to at least two toxophore groups.

Since the completion of this paper an investigation of the chemical constituents of Amanita phalloides has been carried on in the laboratory of Professor J. J. Abel, the report of which will appear shortly. The separation of the two principles described has now been accomplished by chemical means.

## CONCLUSIONS.

From the experiments detailed in this paper it may be concluded that Amanita phalloides contains besides phallin, or the hæmolytic principle of Kobert, another body of toxic nature. Phallin is thermolabile, and is destroyed by the action of pepsin and pancreatin. The other toxic body is thermostable and is resistant to pepsin and pancreatin. The two substances, moreover, possess different toxophoric and haptophoric groups, since an antiserum produced by the immunization of animals to the thermostable body has no neutralizing effects upon phallin.

The thermolable body, phallin, produces the subcutaneous œdema and hæmoglobinuria, and, in virtue of its blood-laking properties, the pigmentation of the spleen. The thermostable body produces hæmorrhage and necrosis, and the fatty degeneration of the parenchymatous organs. The two bodies exist side by side in watery extracts of the fungus, but they cannot be considered as two constituents of a single poison exerting a variety of effects. To the hæmolysin the name phallin has already been given by its discoverer, Kobert. For the thermostable substance described now for the first time, the name Amanito-toxin is proposed provisionally.

# CONCERNING THE MORPHOLOGY AND REPRODUCTION OF SPIROCHÆTA PALLIDA AND A RAPID METHOD FOR STAINING THE ORGANISM.

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PLATES XXI AND XXII.

The object of this paper is to describe a simple method for the staining in smear preparations of Spirochæta pallida and to present certain observations which I have been enabled to make upon the morphology and reproduction of the microorganism through the use of this staining method. I have examined a large number of cases of syphilis and various syphilitic lesions, and it is upon the study of a relatively large mass of material that I base my conclusions. I have purposely omitted references to the literature of my subject as this communication is intended to be a report of personal observations.

The Giemsa staining method, which is based upon the Nocht-Romanowsky principle of staining, is so time-consuming, where the question of a possible quick differential diagnosis is to be considered, as to be quite useless. Besides this, we have to deal with the disadvantage that the organism is stained so lightly as at times to be readily overlooked. One can safely state that there is no modification of the Romanowsky method which will not stain the pallida, but I believe the results to be less brilliant than those obtainable by the method I am about to describe.

I prepare a dye for Spirochæta pallida as follows:

In 200 cubic centimeters of water two grams of lithium carbonate are dissolved and two grams of methylene blue are added. While some other carbonate may be used, the use of lithium salt seems to me preferable. The methylene blue is either Merck's medicinal, or Gruebler's B X, or Koch's rectified. This alkaline methylene blue solution is heated in a rice-boiler with a moderate amount of heat until a rich polychrome has formed. The steps leading up to this formation are followed by taking every few minutes a sample in a test-tube, and examining by holding it against artificial light. As soon as a distinctly red color is obtained the desired degree of heating has been reached. During the cooling, which is allowed to take place spontaneously, more of the polychrome will be formed. The residue which is undissolved is removed from the cooled dye by pouring through cotton in a funnel.

To one half of this alkaline polychrome dye 5 per cent. acetic acid is gradually added until a strip of litmus paper shows above the line of discoloration a distinct acid reaction. The remaining half of the dye is now added, so as to carry the reaction back to a low degree of alkalinity.

A weak eosin solution is now prepared, approximately one half per cent. French eosin, and this is added gradually, while the mixture is being stirred, until a filtered sample shows the filtrate to be of a pale bluish color with slight fluorescence. The mixture is allowed to stand for one day and then filtered. The precipitate which has separated is collected on a double piece of filter paper and dried at room temperature, or at a temperature not exceeding 40° C. Excessive heat injures the dye.

When this precipitate is completely dried, it is readily removed from the filter paper and may then be dissolved without further washing in commercial wood alcohol.[1] The solution should be allowed to stand for a day in an open vessel, and then filtered. An alcohol insoluble residue may be present which is to be removed. The strength of this alcoholic solution is approximately 1 per cent.

To use the stain on slide smears, one drops sufficient dye on an unfixed preparation to cover it, allows the dye to act for three or four seconds, and pours off the excess of dye. The slide is now introduced *slowly* into clean water with the *film side down*, permitting in this manner an interaction between the film of adhering dye and the water. The slide is held in this slanting position in the water for another four or five seconds, and is next shaken in the water so as to wash off the excess of dye.

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[1] Commercial wood alcohol is to be preferred to the pure article, as it gives better fixations.

The reason for holding the preparation stained side downward is to prevent the forming precipitate from falling upon the smear and disfiguring it. A well-stained preparation is obtained in this manner, and the pallida appears of a violet color, which may be changed to bluish-black by flooding the preparation for fifteen or twenty seconds with the ordinary iodo-iodide of potash solution of Gram, washing and drying as usual. This counterstaining with Gram's solution permits a moderate degree of differentiation with alcohol, but this has up to the present offered no advantages, as the pallida decolorizes readily.

My study has embraced sixty cases of undoubted syphilis, and of these only eight have failed to show the pallida. The results depend very much upon the technique employed. Repeated examinations were not infrequently necessary before the parasite could be demonstrated. Moreover, as it could not be demonstrated at all times in every lesion of the same individual, the desirability of making preparations from both primary and secondary lesions located in different parts of the body is evident. The number of pallida found did not seem at any time to bear relation to the severity of the infection. The mere fact that numerous organisms could be obtained one day from a lesion, while on the following day but few were obtained from the same lesion, although the same technique was employed, goes to show that numbers are not always the most important factor in determining success of demonstration. The appearance and grouping of the pallida frequently suggests other possibilities, as I will show presently.

Since I lay much stress upon technique, I will describe the method with which I obtained my best results: curettage was always resorted to with a view to removing surface epithelium and exudate, the latter especially, because the finding of the pallida in smears showing pus proved to be a matter of great difficulty. The curettage was carried sufficiently far to cause the appearance of a small amount of serum and blood, and it was preferably done at the edge of the lesion rather than in the center. The deeper curettages also helped to eliminate other organisms from the field. Wherever it was possible, the making of impression preparations was preferred, not only because of the ease with which they are made, but because they proved to be of advantage in the study of the morphology and the probable mode of reproduction of the pallida. A lesion of the throat had to be handled differently. Gentle curettage usually caused the appearance of a little blood which was removed with a sterile platinum wire and smeared upon a slide; in fact the majority of mouth lesions and those located on the vaginal wall had to be studied in this manner. In the examination of roseola efflorescences the curettage was steadily carried to the bleeding point in half a dozen or more places, and a number of preparations were made, some being taken at the moment preceding the appearance of blood corpuscles, because it was found that the optimum point was readily overstepped when it was a question of finding the spirochætæ rapidly. Under all circumstances the finding of the parasite in these roseolæ was more or less difficult.

Blood smears were frequently taken from the lobe of the ear in the usual manner and examined carefully, especially in those cases where a chancre was of short standing, but this tedious work remained entirely negative as far as finding the organism was concerned. Moist papules seemed to contain the largest numbers, and incidentally it may be stated that in many instances the longest individuals were found in them. The majority of chancres showed almost equal numbers, but only after the curettage had been carried out at the edge of the lesion.

Several cases studied were of exceptional interest. I will state briefly some of the most important features of them.

Case 1.—Female who denied all knowledge of a primary lesion. Body covered with a papulo-macular eruption. On careful examination a small moist papule was found concealed under the left mamma. Slides were prepared from the moist papule and from the eruption on the arm and shoulder. Impression preparations from the moist papule showed the pallida in enormous numbers, many fields showing from twenty to thirty individuals. The macules from the arm and shoulder showed thousands of spirochætæ, but among them pallida forms were not discovered. The types seen were refringens, and other straighter forms.

Case 2.—Male. Papules on scrotum and penis. Macules, general. The former showed after repeated examination a small number of pallida, the latter failed to show pallida. The papules were of four weeks standing.

Case 3.—Female. Examination extending over eight weeks during administration of mercury. Mucous placques were studied. The pallida was found at each examination, but the last two examinations showed diminution in numbers. In this connection another case may be mentioned in which the examination of mucous patches gave on two occasions positive and in the intermediate examination negative results.

Several lesions which were negative were as follows:

Mixed (?) lesion of the penis; double primary lesion of the tongue and penis (uncertain history of infection); tubercular syphilide over the right eye. The last showed the refringens.

Late lesions, believed to be tertiary, were repeatedly examined with negative results.

Control examinations of non-syphilitic lesions were also made. In a case of acne and another of eczema spiral organisms were found, but they could be distinguished from the pallida. They were thicker and the turns less sharp; ends pointed. Smegma gave some suggestive organisms: they were as delicate as pallida but the characteristic sharp turns of the latter were absent. These last organisms when suspended in salt solution and kept at body temperature were highly motile.

In morphology, the pallida which I studied agreed, in general, with the description of Schaudinn and Hoffmann. As regards the question of flagella, I found on occasional organisms what might have been considered such appendages. I examined hundreds of spirochætæ in impression preparations in order to find one organism showing the prolongation. It seemed indeed too thick to be an ordinary flagellum. I was unsuccessful with Loeffler's stain. In the fresh state I could not discern flagella.

The length of and hence the number of curves in the pallida varied greatly. As many as twenty-nine curves have been counted (Plate XXII, Fig. 10), on an individual of uniform thickness. In thickness there is also variation, so that the dimensions given by Schaudinn and Hoffmann do not hold absolutely. (Plate XXI, Fig. 4, a.) The thickness may approach that of refringens; and in a chancre of the lip I found two pallida of unusual length. One possessed forty turns at least, and stretched across the greater part of the microscopic field. It was very thin and faint. I regard the pallida as a spiral of variable length and thickness, attenuated at the ends so as to form a fine point, the terminal end being as a rule no more than one coil in length.

Impression preparations which were dried immediately showed not infrequently organisms of the pallida type which, in places, were rather straight, so that I do not believe the spirochæta to be a rigid spiral; but, on the contrary, I believe it to be very flexible. The living organism presents rather constantly the spiral form, if the medium in which it moves be sufficiently abundant and fluid. I have made it a rule to place a thin hair between the cover-glass and the slide, so that close proximity of the cover may not interfere with the movements of the parasite, and to allow the fluid to enter by capillarity. I prefer such preparations to the hanging drop. The movements of the pallida may be compared to those of the spirochæta of relapsing fever, except that those of the pallida are slower even at room temperature. The pallida seems to move with equal facility slowly backward and forward. It seems to turn on its axis, and while its movements are evident, it appears to remain rather stationary.

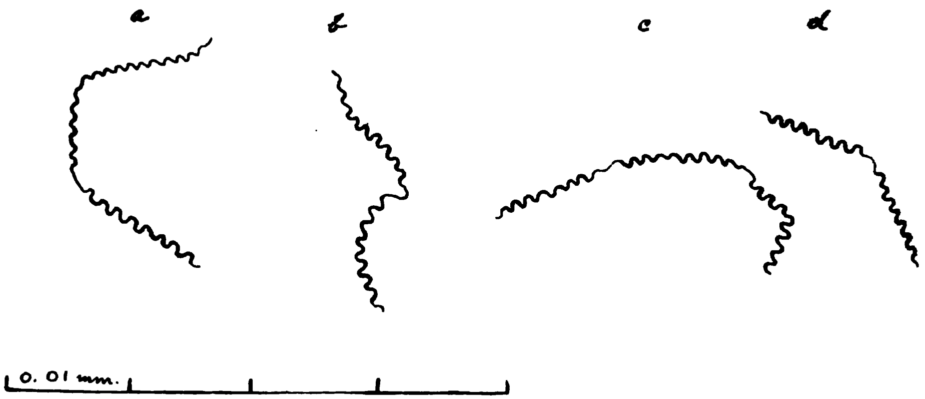
The impression preparations seemed at first to yield information regarding the mode of reproduction of the pallida. At the February meeting of the New York Pathological Society I expressed the belief that longitudinal division occurred, leading to the production of three or four individuals which remained attached end to end, before separation by transverse section. On Plate XXII Figure 4, e, shows, for example, a relatively thick, short parasite which becomes attenuated at one end, from which a second organism seems to arise and split off. Still other organisms are short and thick and give rise, apparently, at one end to several individuals which can be made out distinctly. Moreover, I was able to show in a single field of the microscope ($\frac{1}{12}$ inch immersion lens) as many as five spirochætæ, which on close examination proved to be double, and still other spirals seemed to be uncoiling (Plate XXII, Figure 9). These appearances I at first interpreted as examples of longitudinal division.

I have now come to the conclusion that the intertwining can be accounted for in a simple way by considering the effects of (1) the mode of progression of the organisms, (2) the large numbers which are present in the lesions, and (3) the consistence of the medium in which they move. Of these factors the first one is doubtless the most important.

In order to obtain a better understanding of the appearances suggesting longitudinal division, I prepared two wire models, coiled as the pallida is supposed to be. By carelessly throwing them together a double spiral, permitting of lateral separation, was eventually formed. By rotating one spiral to the right and the other to the left, one lying behind the other, all figures from incomplete to complete apposition, with the production of a thickened spiral, were reproduced. In this condition lateral separation, when one remembers the flexibility of the pallida, is seen to be possible, especially if the individuals moved in opposite directions. What, however, is unaccounted for and, by this view, impossible to account for, is the end to end attachment forming a continuous and long spiral organism with attenuated points in two or three places (Plate XXI, Figure 2).

There was another objection to the view of longitudinal division: the number of turns in the apposed organisms was subject to too great variation. While one organism showed six turns, the other showed as many as ten. This appearance could be more easily explained on the supposition of entanglement.

Not infrequently I noticed spirals of the pallida type which were twice the length of typical specimens. Near the middle a thinned out, more or less straight part appeared, and beyond, on each side, the curves reappeared equally. To account for this appearance as the result of longitudinal division, one might suppose the individuals to have whipped apart, while the two ends remained united. This was the attenuated section. In endeavoring to account for these different appearances on the basis of longitudinal division, I did not leave out of account the possibility of transverse division, which, indeed, I regarded as probably the true mode of propagation. According to this conception the attenuated parts described would represent the points of separation. Further studies have supplied me with unmistakable evidence of transverse division. The length of these specimens of pallida was unusually great, and in the course of one spiral three or even four thinner and straighter portions were encountered. These specimens were found in preparations in which the large numbers present indicated active multiplication. The attenuations occurred at intervals of six to eight curves. It is difficult to account for this appearance upon any other supposition than transverse division. Admitting this as the probable mode of division, it should be said that not infrequently the product is greater than two individuals at one division; I have seen a dividing spiral which apparently was yielding four individuals.



**Transverse division of Spirochæta pallida. Camera lucida drawings.**
- **a. Organism dividing to form probably three; curves and diameter irregular.**
- **b. Characteristic division.**
- **c. Division into three; organisms irregular.**
- **d. Division into equal organisms.**

Koch[2] has just published an account of the study of the spirochætæ of recurring African fever and his figures show unmistakable evidence of transverse division.

These figures show so great a similarity to those which I obtained from Spirochæta pallida that the two organisms may, I think, be regarded as multiplying in the same manner. Other points of similarity between the recurrens and the pale spirochætæ are the occurrence of thick individuals and the tendency to form masses where the organisms are sufficiently abundant and the medium suitable.

It must, however, be considered as still a question whether some of the thick and uniform types of pallida represent two accidentally intertwined individuals. It does not seem impossible that conjugation may be going on in these forms. And if this were true, then the intertwinings and the zooglea-like formations would not represent accidental conditions, but unions looking to renewal of life by the fusion of two elements. The remaining possibility, which is not to be excluded, is that the thick forms are involutions. I have seen undoubted examples of involutions in short, thick pallida.

I am deeply indebted to Professor E. K. Dunham for many helpful suggestions which led me to definite conclusions regarding the mode of division of the pallida. Further work is now being carried on largely along lines suggested by Professor Dunham, who has taken an active interest in this work and has placed the facilities of the laboratory at my disposal. I wish also to express my gratitude to the Dean of the College, Professor Egbert Le Fevre. While the number of gentlemen who have favored me with cases, syphilitic and others, upon which my work is based, is too great to permit of individual mention, I do not wish to fail to acknowledge my special indebtedness to Dr. Flexner of the Rockefeller Institute who has been ever ready to help and suggest, to Professor Fordyce and his staff of the Dermatological Department, and to Professor van der Poel and his staff of the Department of Genito-Urinary Diseases. Had it not been for the courtesy of these gentlemen, I should have been without the most valuable material.

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[2] Koch, *Berl. klin. Wochenschr.*, 1906, xliii, 185.

## EXPLANATION OF PLATES

### PLATE XXI.

(All Spirochæta pallida.)

Fig. 1.—(*a*) Division into three individuals.
(*b*) " " two "
Fig. 2.—Division into three individuals, red blood corpuscles in the field.
Fig. 3.—(*a*) Very long pallida; atypical curves.
(*b*) Long pallida, partly double and thicker than normal.
(*c,c,c*) Typical specimens of pallida.
Fig. 4.—(*a*) Single organism, abnormally thick.
(*b*) Division into two organisms.
(*c*) Typical pallida.
Fig. 5.—Massing of spirochætæ; some blood corpuscles in the field.
All figures from moist papules; original amplification × 2200; reduced ¼.

### PLATE XXII.

Fig. 1.—(*a*) Spirochæta pallida; close intertwining of two individuals.
(*b*) Long pallida; only partly shown. (× 1800.)

Fig. 2.— Very long pallida; curves partly atypical; evidence of transverse division; a second short organism intertwined at one end. Red corpuscle near center. (× 1800.)

Fig. 3.—Spirochæta pallida; division into three. (× 1800.)

Fig. 4.—(*a* and *b*) Typical pallida.
(*c* and *d*) Atypical curves in pallida.
(*e*) Thick pallida apparently splitting into three.
(*f*) Two pallida spirochætæ, partly so closely coiled as to resemble thick part of *e*. (× 1800.)

Fig. 5.—Apparently single pallida, attenuated at each end. (× 2200.)

Fig. 6.—Pallida with *straight* central body. (× 2200.)

Fig. 7.—Spirochæta refringens with membrane and blunt projection at one end which stains blue while the body is deep purple in color. (× 1800.)

Fig. 8.—(*a*) Spirochæta refringens; blunt projection as in Fig. 7.
(*b*) Spirochæta plicatilis, probably two individuals closely applied. (× 1300 approx.)

Fig. 9.—Spirochæta pallida; three double organisms of which A is irregularly intertwined; B and C show at points indicated by arrows perfect apposition of coils. (× 1800.)

Fig. 10.—(A) Spirochæta pallida, 7 coils.
(B) " " 29 "
(× 1800.)

Figs. 1, 2, 3, 4, reduced ⅛.
Figs. 1, 2, 3, 4, 5, 9, from a moist papule under breast.
Figs. 7 and 8 from mucous patch.
Fig. 10 from a chancre of lip.

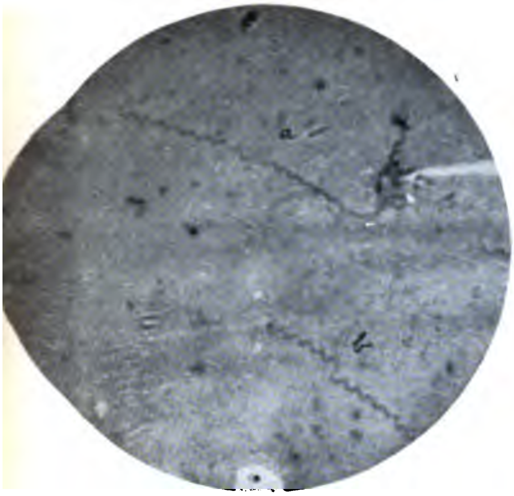

Fig. 1.

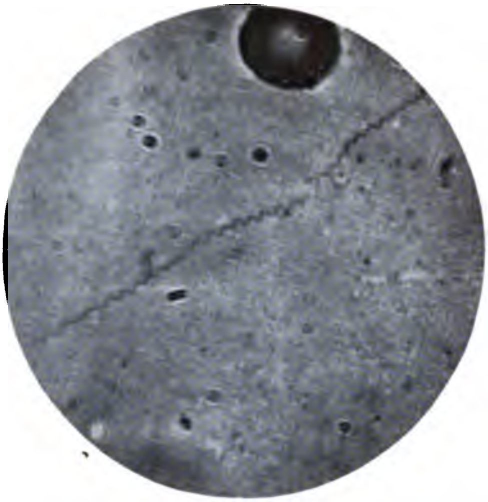

Fig. 2.

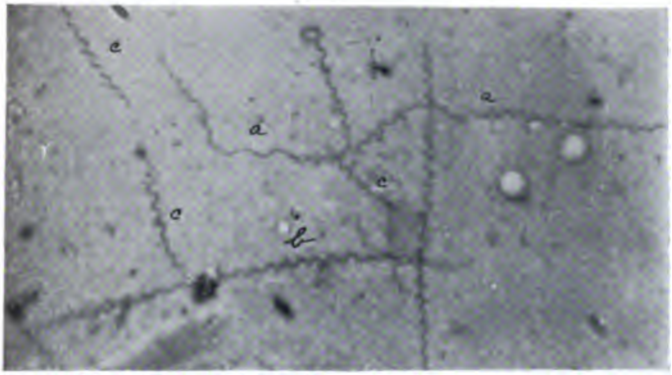

Fig. 3.

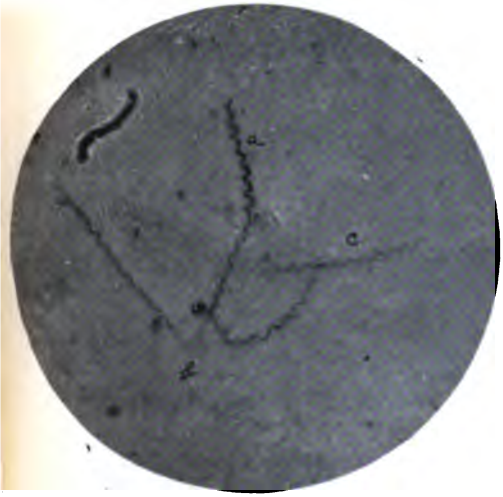

Fig. 4.

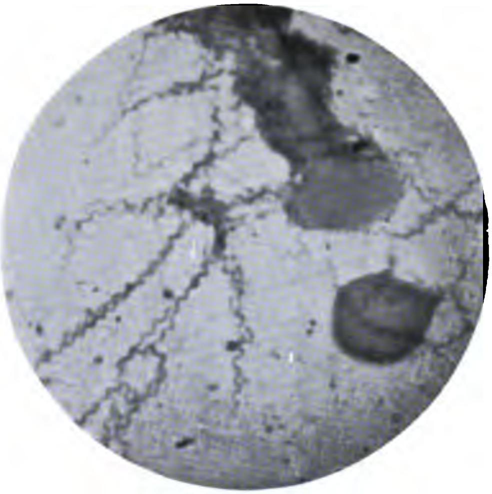

Fig. 5.

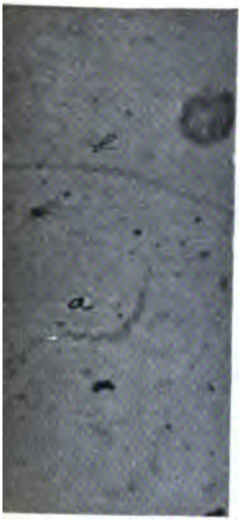

Fig. 1.

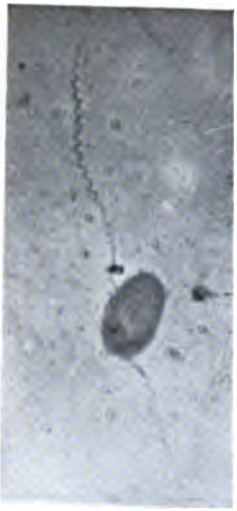

Fig. 2.

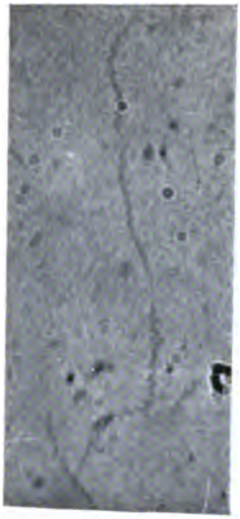

Fig. 3.

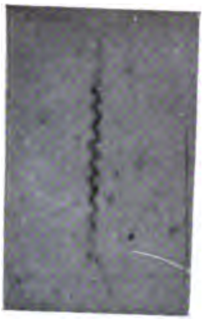

Fig. 5.

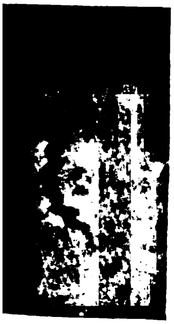

Fig. 6.

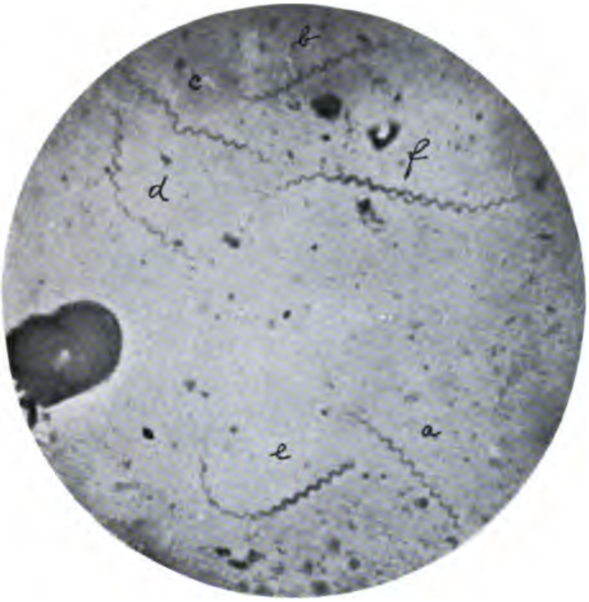

Fig. 4.

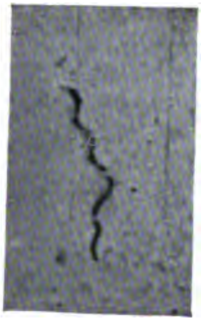

Fig. 7.

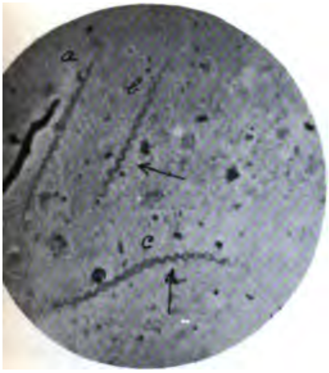

Fig. 9.

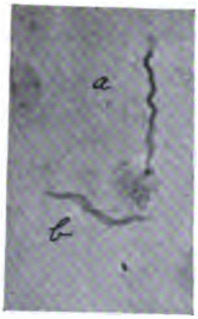

Fig. 8.

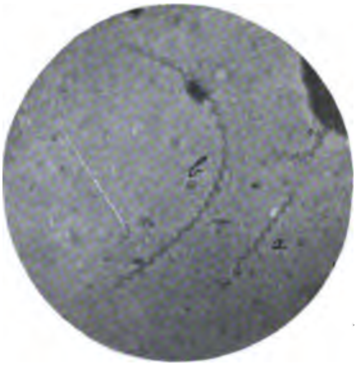

Fig. 10.

# ON GLYCYLPROLIN ANHYDRIDE OBTAINED ON TRYPTIC DIGESTION OF GELATINE.

BY P. A. LEVENE AND W. A. BEATTY.

*(From the Rockefeller Institute for Medical Research, New York.)*

In a previous communication on tryptic digestion of gelatine one of us (Levene) reported the finding of a substance having the composition $C_7H_{10}N_2O_2$.[1] The mode of separation of this substance was fully described in an article published by G. Wallace and one of us (Levene [2]). In the previous publications the supposition was expressed that the substance might be a precursor of prolin. Indeed on further cleavage with mineral acid the substance $C_7H_{10}N_2O_2$ decomposed into prolin and glycocoll, thus showing that it is a dipeptid, prolylglycin anhydride—$C_5H_9NO_2 + C_2H_5NO_2 - 2H_2O = C_7H_{10}N_2O_2$.

One and five tenths grams of the air dry substance was heated for five hours with strong hydrochloric acid in a sealed tube at 150° C. The product of the reaction was taken up in water and evaporated to dryness under diminished pressure, and the residue again dissolved in water, and evaporated. This operation was repeated three times. The remaining hydrochloric acid was removed by means of silver sulphate, and the sulphuric acid by means of barium hydroxide, and the filtrate from the barium sulphate was evaporated to dryness. The residue was crystalline in nature, possessed a very sweet taste, formed with copper oxide a salt, gave rise to pyrrol on sublimation, and, on heating, gave the odor of pyrolidin. Thus the presence of prolin in the residue was made very probable. The presence of glycocoll was demonstrated by the formation of the typical picrate described by one of us (Levene).

The separation of the two aminoacids was accomplished by means of boiling methyl alcohol. The mixture was repeatedly extracted with methyl alcohol until the residue no longer gave on sublimation the pyrrol test. The substance gave a bright pink sublimate which is characteristic for glycocoll formed a picrate resembling that of glycocoll, and possessed a very sweet taste. On rapid heating it turned brown and began to melt at 240° C., and decomposed at 250° C. (An Anschutz thermometer was used for the experiment.) The substance had the following composition:

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[1] *Journal of Exper. Med.*, 1906, viii, 180.

[2] *Zeitschr. f. physiol Chem.*, 1906, xlvii., 143.

0.0881 gram of the substance gave on combustion 14.4 c.c. nitrogen (over 50% KOH) at T° = 18.5° C and p = 747 m.m.

For $C_2H_2NO_2$ the quantities are as follows:

| Calculated. | Found. |
|---|---|
| N = 18.66% | 18.87% |

Thus the substance was glycocoll. The yield of it was 0.350 gr.

The methyl alcohol extract was concentrated under diminished pressure and then evaporated nearly to dryness on a water bath. It was then taken up in absolute alcohol, filtered, and dried. The substance had all the properties of prolin. Its melting point was 205° C.

0.185 gram of the substance gave on combustion 20.8 c.c. nitrogen (over 50% KOH) at T° = 22° C. and p = 760 m.m.: N = 13.03%.

For $C_5H_9NO_2$ the quantities are as follows:

| Calculated. | Found. |
|---|---|
| N = 12.17% | 13.03% |

Thus this sample consisted of prolin still containing some glycocoll.

The alcoholic mother liquid was allowed to evaporate over sulphuric acid in vacuum desiccator. A crystalline deposit was formed. It was filtered, washed with alcohol and ether, dried, and analyzed:

0.1356 gr. of the substance gave on combustion 0.2600 gr. $CO_2$. The water absorption tube was lost through an accident. For $C_5H_9NO_2$:

| Calculated. | Found. |
|---|---|
| C = 52.17% | 52.28% |

0.1037 gr. of the substance gave on combustion 0.1995 gr. $CO_2$ and 0.740 gr. $H_2O$. For $C_5H_9NO_2$:

| Calculated. | Found. |
|---|---|
| C = 52.17% | 52.46% |
| H = 7.82% | 7.92% |

This finding furnishes conclusive evidence, lacking up to now, that prolin is a primary component of the proteid molecule.

# ON THE PHOSPHOTUNGSTATES OF CERTAIN AMINOACIDS.

By P. A. LEVENE and W. A. BEATTY.

(*From the Rockefeller Institute for Medical Research, New York.*)

In a communication to the Chemical Society[1] one of us reported on the property of aminoacids to form insoluble phosphotungstates. In a later publication[2] the writers showed the degree of precipitability of aminoacids by means of phosphotungstic acid, and stated that a separation of aminoacids may be accomplished by means of this reagent. The attempt was made to apply the method to the study of the acids resulting from the hydrolysis of gelatine by means of mineral acids. Unfortunately, through an accident in the laboratory, the greater part of the material for this study was lost, and hence in this place there will be reported a method of the separation of oxyprolin and of an acid having the composition $C_7H_{14}N_2O_2$.

*Oxyprolin.*—This communication is prompted partly by a statement made by Emil Fischer in a recent address delivered to the German Chemical Society in Berlin.[3] Fischer points out that considerable difficulty is experienced in obtaining oxyprolin from the mixture of the products resulting from hydrolysis of proteids. The process employed by Fischer consisted in removing most of the aminoacids first by esterification, then by crystallization, and finally by means of phosphotungstic acid. From the mother liquid, remaining after all these precipitations, oxyprolin was separated.

We have observed that oxyprolin can be isolated as a phosphotungstate without great difficulty. It precipitates in the form of an oil which crystallizes on standing and is soluble in an excess of the reagent. Its separation was accomplished in the following manner:

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[1] Jan. 5, 1906; see *Science*, 1906, p. 347.

[2] *Zeitschr. f. physiol. Chem.*, 1906, xlvii, 149.

[3] *Berichte d. deut. Chem, Gesellschaft*, 1906, xxxix, 597.

The product of hydrolysis (after the mineral acid has been removed in the usual manner) was concentrated to a thick syrup, acidulated with sulphuric acid, and treated with a concentrated solution of phosphotungstic acid (4 to 1) so long as an oily precipitate was formed. The supernatant liquid was decanted and the oil treated with an excess of the phosphotungstic acid solution until the oily character of the precipitate disappeared. A part of the oil passed into solution and another part formed a precipitate with the excess of the reagent. The soluble part contained the oxyprolin. On concentration of the solution (after the phosphotungstic acid was removed) leucin first crystallized. The mother liquid from this acid was further concentrated, after which a second precipitate formed. This substance was removed by filtration, washed with alcohol and ether, dried, and analyzed. The crude substance had the following composition:

0.1271 gram of the substance gave on combustion 0.2242 gram CO$_2$ and 0.1033 gram of H$_2$O; C=48.09%, H=9.03%.

It was then redissolved in little water and two volumes of absolute alcohol were added. A precipitate appeared consisting of typical crystals of oxyprolin. The crystals tasted very sweet and formed pyrrol on sublimation. The melting point was at 270° C.; the composition was as follows:

0.1342 gram of the substance gave on combustion 0.2266 gram CO$_2$ and 0.0843 gram H$_2$O. Hence for C$_5$H$_9$NO$_3$

| Calculated. | Found. |
|---|---|
| C =45.80% | 46.05% |
| H= 6.87% | 6.9% |

From this analysis the substance is oxyprolin.

*On the Substance* $C_7H_{14}N_2O_5$.—This substance was obtained from the fraction of the products of hydrolysis consisting chiefly of glycocoll. It had been stated in a previous communication that glycocoll and alanin under favorable conditions may be separated nearly quantitatively by means of phosphotungstic acid. These phosphotungstates possess typical crystalline forms and are slightly soluble in an excess of the reagent. A phosphotungstate of this nature was obtained from the products of hydrolysis of gelatine. The glycocoll was removed in the form of a picrate. From the mother liquid of the glycocoll picrate, the picric acid was removed in the usual manner, and the solution concentrated to a very small volume and then treated with an excess of absolute alcohol. An oily sediment was formed which on standing was beaded with granular crystalline deposits, and similar deposits were formed on the walls of the vessel. The substance possessed a very sweet taste, gave on sublimation pyrrol, had a melting point of 236° C., and yielded the following composition:

0.1854 gram of the substance gave on combustion 0.2772 gram $CO_2$ and 0.1196 gram $H_2O$; $C = 40.76\%$, $H = 7.17\%$.

In another experiment the substance was obtained in an identical manner and the attempt to purify it by recrystallization was made. For that purpose it was redissolved in little water and treated with alcohol. But as in the course of twenty-four hours no crystal formation appeared, a great excess of alcohol was added. An amorphous precipitate was formed, having a melting point of 232° C. and the following composition:

0.2468 gram of the substance gave on combustion 0.3710 gram $CO_2$ and 0.1880 gram $H_2O$; $C = 40.99\%$, $H = 7.13\%$.

In a third experiment the substance obtained in the same manner was not absolutely free from glycocoll and it contained 37.00% of carbon and 8.00% of hydrogen. This substance was then extracted with a great excess of methyl alcohol. A part passed into solution. The insoluble part was pure glycocoll. It was then attempted to purify the bodies of the first and the second experiment by means of methyl alcohol. It was, however, found that they were completely dissolved in an excess of the reagent. The combined methyl alcoholic solutions were concentrated to a small volume and precipitated with aceton. The precipitate thus formed was amorphous and not absolutely white. It was therefore extracted with a very small quantity of boiling methyl alcohol, washed with alcohol and ether, dried in the xylol bath, and analyzed. It had the following composition:

0.1682 gram of the substance gave on combustion 0.2469 gram $CO_2$ and 0.1077 gram of $H_2O$; $C = 40.75\%$; $H = 7.12.\%$

0.149 gram of the substance gave on combustion 17.5 c.c. (over 50% KOH) Nitrogen at $T° = 22.5°$ C. and $p = 775$ m.m.: $N = 13.73\%$.

The remaining part of the substance was dissolved in a large excess of boiling absolute alcohol. It all went into solution leaving practically no residue. The excess of alcohol was then removed under diminished pressure and the solution further concentrated on a water bath. A crystalline deposit consisting of prismatic needles was formed. In an aqueous solution it reacted acid to litmus, possessed a sweet taste, and had a melting point of 236°–240° C. On sublimation pyrrol was given. An analysis gave the following composition:

0.1214 gr. of the substance gave on combustion 15.00 c.c. nitrogen (over 50% KOH) at T° = 25° C. and p = 763 m.m.; N = 14.25%. For $C_7H_{14}N_2O_5$:

| Calculated. | Found. | | | |
|---|---|---|---|---|
| | Sample I. | Sample II. | Sample III. | Sample IV. |
| C = 40.75% | 40.76% | 40.99% | 40.75% | |
| H = 6.8% | 7.17% | 7.13% | 7.12% | |
| N = 13.54% | 13.33% | | 13.73% | 14.25% |

Thus it was impossible by means of the various methods to alter the composition of the substance, and it is, therefore, probable, that the analyzed body was not a mixture but an individual substance of the composition $C_7H_{14}N_2O_5$. The substance may perhaps be a homologue of Fischer's acid $C_{12}H_{24}N_2O_5$.

# A STUDY OF EPIDERMAL FIBRILS.

BY R. L. THOMPSON, M. D.

(*Associate Professor of Pathology, St. Louis University.*)

(*From the Pathological Laboratory, St. Louis University Medical Department*).

PLATE XXIII.

When the epidermis* is appropriately stained delicate fibrils can be seen in the stratum filamentosum. These fibrils go out from the protoplasm of the cells in different directions and across the intercellular lymph spaces to enter neighboring cells, so that all the cells of this layer are bound together by a delicate fibre system. The departure of the fibrils from the cells gives them the appearance of a thorn-like border whence they have derived the name "prickle" cells. These fibrils, which have been termed intercellular bridges, present in their middle part a slight enlargement, which is known as the Ranvier-Bizzozero nodule. They are not confined wholly to the stratum filamentosum but also may be seen joining together many of the basement cells. In addition to these connecting fibrils there may be seen in the stratum germinativum longer, thicker, more deeply staining protoplasmic fibrils, which run parallel to the long axis of the cells. These fibrils frequently are distinctly spiral in form. They may follow the contour of the nucleus and be wholly within the cell or they may lie at the edge of the cell or in the lymph space between the cells. They appear to be wholly distinct from the fibrils first described. In the normal epidermis these latter fibrils are few and they may be wholly absent in many sections. It is in certain pathological conditions, however, that these fibrils assume importance and offer the basis for the present study.

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* In the human epidermis seven layers are recognized by Ehrmann and Fick (*Einführung in das mikroskopische Studium der normalen und kranken Haut.* Wien, 1905). From below upward they are designated as follows: 1. Stratum germinativum. 2. Stratum filamentosum. 3. Stratum granulosum. 4. Stratum intermedium. 5. Stratum lucidum. 6. Stratum corneum. 7. Stratum disjunctum. The lower cells (stratum germinativum) are placed perpendicular to the corium; they have large, oval, vesicular nuclei with considerable chromatin; the shape of the cells varies from that of a cube to that of a slender high cylinder; they are closer together than in the layer above but are separated by narrow lymph channels. This layer passes insensibly into the stratum filamentosum, the cells becoming larger and more polygonal in shape; the nuclei are larger, rounder, and do not stain so deeply as the nuclei of the basal cell row. The channels between the cells are wider than below. Above this layer the cells are flatter and show granules of kerato-hyalin and finally become fused together and disintegrate. They need not be further described as only the lower two layers are to be considered in this paper.

Among the numerous articles that have appeared concerning these fibrils only the more important need be noted.

In 1863, Schroen[1] described in the rete Malpighii of the epidermis "scalariform striæ," which he considered a part of the cell membrane "with pores to let in nourishment." Max Schultze,[2] in 1864, observed "projections" from the cells of the mesial zone of the epidermis and was the first to apply to these cells the name of "prickle (*Stachel*) cells." He believed that these spines "gripped one another as bristles do when two brushes are pressed together." An important contribution was made by Bizzozero[3] (1871), who first noted the minute thickenings or nodules on these spines. These nodes he considered to be the point of union of two fibrils "welded together end on end." Ranvier[4] (1879) also studied these fibrils and said, "The cells of the rete Malpighii are not individual but are united by protoplasmic filaments. Each of these filaments is the result of the union of two filaments end on end, and the nodule is the result of their union." The nodule Ranvier further considered to be an elastic organ permitting easy enlargement of spaces destined for circulation of lymph between the cells. In 1882, Ranvier[5] published a second article, in which he described these fibrils as "filaments of union absolutely continuous," instead of being two fibrils united end on end.

Up to this time all discussion as to epithelial fibres had centred about these protoplasmic "bridges" or "spines" of the rete Malpighii. In 1889, Herxheimer,[6] by aid of the then new Weigert fibrin stain, described a new variety of fibril which he found running parallel to the long axis of the cells of the lower layers of the epithelium (stratum germinativum). He did not reach a conclusion concerning the significance of these fibrils, but Eddowes,[7] working in Unna's laboratory, came to the conclusion that these Herxheimer fibrils were only fibrin-filled lymph spaces. A year later, Kromayer[8] turned his attention to these fibrils. He used a modification of the Weigert stain which apparently resulted in a better picture than Herxheimer had obtained. Kromayer considered these fibres to be true protoplasmic fibrils, and not artifacts. In addition to the Herxheimer fibrils he also described a special fibril (*Haftfaser*) extending into the corium and possibly serving to join the corium to the epidermis. Some of the fibrils Kromayer described as having a corkscrew shape.

Kromayer's views were strongly opposed by various investigators. Beneke[9] believed these fibrils to be only a part of the cell membrane. Schütz[10] considered Kromayer's fibres to be only the well-known "Ranvier" fibres altered by the staining method used. This author also considered Herxheimer's fibres to be an artifact. In the same year Herxheimer and Müller,[11] by aid of Weigert's neuroglia stain, reached the conclusion that these basal fibres represented the cuticular membrane of the cells and that the fine protoplasmic fibrils might be differentiated protoplasm.

Rabl[12] (1897) investigated these fibrils and considered the spiral form of the Kromayer fibrils an artifact. Rabl believed that the fibrils were formed by the coalescence of numerous finer fibrils which through shrinkage became a compact, broad fibre. Kromayer,[13] in a later paper, defended the "corkscrew" fibre, which he considered a distinct fibril in no way to be considered a part of the cell membrane. Herxheimer[14] also devoted a later paper to these fibrils which he described as being produced from a certain specific part of the cell protoplasm.

Unna[15], who has contributed several articles on these fibrils, in a recent paper devoted in great part to a consideration of a new stain gives us some excellent pictures of the various fibrils of the epidermis. Unna believes that the Herxheimer and similar fibrils are a part of the general fibre system.

It will be seen from the foregoing that there is still considerable dispute regarding the nature and significance of these fibrils. Although at first some observers considered the basement fibrils as artifacts, the later observers agree that they are true fibrils. The spiral form, however, that certain fibres assume is considered by some artificial. There is a dispute as to whether the fibres are a product of the protoplasm of the epithelial cells. By some they are considered merely the differentiated cuticular membrane, by others a condensation of the fibrillar ground substance of the cells. Moreover, it is a disputed question whether these fibrils are all a part of one general system, or whether the various fibrils are to be considered separate and distinct. While the fibrils of the basement layer are described in conditions incident to epithelial proliferation there seems to be no stress laid on the relation of fibre production to cellular activity.

The different views regarding these fibrils may be accounted for, in part at least, by the somewhat complicated technic by which they have been studied. Previously the Weigert fibrin stain or some of its modifications, such as the Kromayer stain, or Unna's method have been used for their demonstration. All these methods are, however, extremely complicated, and admit of good results only after considerable practice. Fischel[16] has recently described a modification of the Kromayer stain that shortens the process somewhat, but is still far from simple.

For the demonstration of these fibrils the phosphotungstic acid hematoxylin stain,* as described by Mallory[17] for fibroglia fibrils, was used. Two methods were employed in the examination of tissues, as follows:

*Method A.*—1. Fix in Zenker's fluid; small pieces of skin are stretched out on filter paper and placed in the fluid immediately after excision.

2. Transfer celloidin or paraffin sections from water to a one-fourth per cent. aqueous solution of permanganate of potassium for from five to ten minutes; wash in water; place in a five per cent. solution of oxalic acid for from fifteen to thirty minutes; then wash thoroughly in water.

3. Stain in phosphotungstic acid hematoxylin for from twelve to twenty-four hours; wash quickly in water; dehydrate in alcohol; clear in xylol, and mount in xylol balsam.

*Method B.*—1. Fix freshly excised pieces in four per cent. formaldehyde (ten per cent. formalin). 2. Stain frozen sections, without mordanting, in phosphotungstic acid hematoxylin for from thirty minutes to four hours.

3. Immerse in water, alcohol, xylol, and xylol balsam as before.

For routine study the Zenker-fixed material was used and in most cases both paraffin and celloidin sections made. By this method the epidermal fibrils are stained a deep blue. The intercellular connective-tissue fibrils of the corium are pale brownish red. The nuclei of the cells are stained blue.

In addition to the sections that were prepared as above, frozen sections were made by Method B and carried along for the purpose of comparison. These sections were a considerable help in the study. When properly stained by this method everything in the section with the exception of the epidermal fibrils is a pale brownish red, while the fibrils are a deep blue. This gives a contrast that is not obtained by Method A. The objection to this method is, however, that at times there is inadequate fixation, and therefore loss of fibrillar structure, which the Zenker-fixed material does not suffer. I can account for this only by the fact that the quality of commercial formaldehyde solutions cannot be depended on. If one could feel certain of the fixation, the simplicity of this technic, the quicker action of the stain, and the better contrast in the sections, would make it commend able. It is at any rate essentially practical for a rapid demonstration of epidermal fibrils in freshly excised tumors of the skin.

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* It is necessary to use a well ripened stain to get good results. Mallory suggests the addition of an oxidizing agent, such as a small amount of peroxide of hydrogen, if the solution does not stain. In this work a stain somewhat over a year old was used. The staining time, as given here, may have to be prolonged if a less intense stain is used.

In the course of variola diagnosis in which both the above methods were employed, these fibrils of the epidermis were first noted. This simple technic, which seemed to obviate as far as possible all artifacts and to permit a fairly strict construction of the pictures obtained, seemed so well adapted to their study that a systematic effort was made to obtain various skin sections, which might offer some points of interest to our knowledge of the epidermal fibre system. In common with many other histologic and pathologic problems it was assumed that a preliminary study of these fibrils in animals would be of service in their final interpretation. Herxheimer (*loc. cit.*) has stated that in the animals which he examined (rabbit, rat, and guinea-pig) the long fibrils of the basal cells were wanting.

By permission of Mr. Frank Hagenbeck and by assistance of Dr. R. B. H. Gradwohl, to whom I wish to express my indebtedness, I was enabled to obtain skin sections from a number of the animals in the Hagenbeck zoological collection. Sections of skin from twelve animals were examined.

In regard to the cells of the epidermis of these animals it may be said that usually the different layers as described in human epidermis can be made out. There may be, however, a poor development of any one of the layers. Some animals show a sudden transition from stratum germinativum to stratum granulosum, with little development of the polygonal cells of the stratum filamentosum. Others show the polygonal cells extending to the corium with only occasional suggestion of cells of a columnar type in the stratum germinativum. In regard to the fibres, however, there is a considerable divergence, especially in certain of the animals, from the picture seen in human epidermis. In all the animals excellent pictures are obtained of the connecting fibrils of the stratum filamentosum. They appear more prominently than they do in similarly stained sections of human epidermis. It is in the stratum germinativum that the difference between animal and human skin and among the skins of the various animals examined is to be noted. This difference is in the development of the fibrils of the basement layer and therefore a separate description of these fibrils follows:

*Kangaroo* (*Macropus major*).—Only an occasional fibril is seen, extending from the corium to the upper pole of the nucleus in one of the lower rows of cells. The cells of the basement row are not distinctly columnar in this animal.

*Puma* (*Felis concolor*).—Basement fibrils are rarely seen. Epithelial cells are flattened and the epidermis is only two or three cell layers deep. This skin was taken from a young animal.

*Tapir* (*Tapirus indicus*).—There is better development of the cells than above, and quite a number of short, sometimes wavy, fibrils are present in and between cells of the basement row.

*Fat-tailed sheep* (*Ovis aries steatopyga*).—The epidermis here is poorly developed, but an occasional wavy fibril may be seen, usually lying at the edge of the cell.

*Water deer* (*Hydropotes inermis.*).—Several fields in the section show groups of the fibrils both within and at the edge of the lower cells. Occasionally a fibril extends into the second cell row.

*Guanaco* (*Auchenia huanaco*).—In general the fibrils are absent, but about the hair follicles, especially, occasional well developed fibrils are seen. One cell in mitosis shows two fibrils embracing the nucleus on either side.

*Angora* (*Capra ancyrana*).—In this skin numerous fibrils can be seen both in and between the cells of the basement row, some extending into the corium and some extending into the mesial zone of flattened cells. Many of these fibrils are of a distinct corkscrew shape. They lie for the most part at the edge of the cells. Some are in lymph spaces between the cells.

*Camel* (*Camelus dromedarius*).—This skin was taken from a young animal and shows fair fibre development. The fibrils are seen only in certain parts of the section. The lower cells are usually flat rather than columnar, and certain fibres can be seen running parallel to the corium instead of perpendicular to it as is usually the case.

*Axis deer* (*Rusa indica*).—In certain areas a few fibrils are seen, rarely extending above the second cell row. The fibrils here lie for the most part at the cell edge.

*Llama* (*Auchenia llama*).—There is even better development here of the basement fibrils than in the section last described. The fibrils are practically everywhere present but certain areas show especially thick grouping. Some of the fibres are quite broad, and are for the most part straight, or rather, lightly curved, in conformity with cell contour. They appear most frequently at the edges of the cells.

*Zebra* (*Equus montanus*) (Fig. 1).—Here the fibres are still more prominent and quite generally distributed. They may be seen both within and at the edges of the basement cells. Some of the fibrils run through the cells of the second row and end in the third row of cells. Very distinct spiral and corkscrew forms may be seen.

*Elephant* (*Elephas africanus*) (Fig. 2).—The cells of the epidermis are here somewhat crowded together, due apparently to the pressure of the thick, horny layer. The basement fibrils are everywhere present and more strongly developed than in any of the other sections. Where these fibrils approach the cells above there is a suggestion of their unity with the fibrils of this layer which will be discussed later on.

Fibrils appear in the lower cells of the epidermis in certain animals and in general vary in length and thickness. They lie usually at the edge of the cell, appearing at times as a thickened cell membrane. In places they are more or less detached from the cell. They are usually straight or slightly curved, but sometimes assume a distinct spiral form. The lower ends of the fibrils may be in contact with the corium. Sometimes a fibril extends into the corium for a short distance. There is no evidence, however, that these fibrils serve in any way to join the epidermis to the corium. The fibrils are usually limited to the length of the lower row of cells, but sometimes extend well up into the second cell row. In all these animals, excellent pictures are obtained of the fibrils of the stratum filamentosum ("bridges").

I have also found in the skin of Necturus well developed spiral fibres almost completely surrounding the nucleus of each cell of the basement row (Fig. 3). These fibrils are sometimes seen in the cells of the next higher row, but not so well developed. They are situated well in the protoplasm of the cell in the lower row and approach the periphery of the cell when seen in the next row. While there are no "bridges" in the skin of Necturus, these fibres seem to differentiate themselves into a substance that joins together the cells of the upper layers of the epidermis.

Returning to human skin, the fibrils joining together the cells of the stratum filamentosum in conditions apart from normal will first be considered. Increased distinctness of these fibrils may be seen in human epidermis where the cells are somewhat separated by fluid, as in varying conditions of œdema. In such situations these fibrils may be seen joining together the cells, not only in the stratum filamentosum, but in the stratum granulosum above and the stratum germinativum below. That is, in situations that admit of a proper view of all the cells of the epidermis they are seen to be joined together from top to bottom by delicate fibrils that extend some distance into the cells.

The histologic importance of these fibrils, which are so prominent in both human and animal epidermis, may be emphasized by the fact that I have found them reproduced in skin islets of teratomata exactly as they appear in normal epidermis.

In addition to these fibrils, which are commonly termed the ntercellular bridges, there may be seen in the conditions of œdema above described, especially if the section is taken somewhat distant from the edge of a granulating wound of the skin (Fig. 4) or from the edge of a vesicular lesion such as is found in variola and vaccinia, in addition fine or coarse fibrils, intracellular or in part free from the cell, that are not noticeable in the normal epidermis. These larger fibrils run perpendicularly to the corium and sometimes extend a short distance into it. Some of them are quite long, extending into the second or third row of cells. The end to end juncture of certain of the cells of these rows is seen to be affected by these fibrils. The appearance of these fibrils in this situation and their distribution to the cells above the basement row suggests a unity of the two apparently separate sets of fibrils that have been described.

This suggestion of the unity of these apparently different fibrils above noted, is emphasized in a section taken from the new growing epithelium near the ulcerating edge of an epithelioma of the lip where there is very rapid epithelial proliferation (Fig. 5). This section shows in the stratum germinativum a luxuriant growth of the long protoplasmic fibrils. Not all these fibrils, however, are in the protoplasm. From the rapid pushing up of the epithelium many are freed from the cell, either wholly or in part, and lie in the space between the cells, or lie just at the edge of the cells as an apparent membrane. Some of the fibrils are quite long, extending upwards a distance of several cells. Occasionally a fibril is seen extending a short distance into the corium. A considerable number of the fibrils have the spiral form that is frequently described. In the stratum filamentosum we find numerous cells joined together by these fibrils. Some are joined end on end but frequently the fibrils deviate and form also a side to side juncture, or again they partially deviate and join the end of one cell to the side of another. Where these fibrils have fully differentiated themselves into the connecting fibrils of the stratum filamentosum they are longer, more irregular, and thicker than the "bridges" in normal epidermis. This is to be expected in newly formed tissue where compensatory growth is always a hypercompensation at first. Moreover, these fibrils do not show the central nodes (Ranvier-Bizzozero nodules) that are prominent later on, and this fact would suggest that the nodes were incident to the shrinkage of the fibres. Everywhere in the section there is the irregularity which is evidence of youth and is comparable to the irregularity of young connective-tissue fibrils. The evenness possessed by structures further removed from adolescence has been attained in no place in the section.

The proof of the unity of these fibrils that is evident in this situation is not confined wholly, however, to situations that show separation of the cells, but may be found under opposite conditions, namely with compression of the cells incident to increased cornification, occurring in association with more or less rapid proliferation of epithelium. Papillomata of the skin (warts) offer material for the further consideration of these fibrils

Under pressure from the increased development of the stratum corneum (Fig. 6) the fibrillar groundwork of the epithelium is very conspicuous. There is only slight separation of the cells and consequent shortening and crowding together of the connecting fibrils. The perpendicular fibrils are very distinct and are best seen in the basement row of epithelial cells where they present a brush-like border to the corium. They are not limited to the lower row or rows of cells, but may also be seen extending into the layer of polygonal cells where they either continue as perpendicular fibrils or branch off and become the so-called "bridges" of this layer. The unity of the fibre system is everywhere apparent.

Still greater degrees of compression emphasize the fibrillar structure of the epidermis but may be too great to admit a close following of the fibrils. Such a section is seen in epidermis taken from the thickened palm of a laborer (Fig 7) following amputation for extensive phlegmon of the arm. Instead of distinct cells connected by fibrils there is an apparent moulding together of the cell protoplasm into a solid mass. in which the situation of the nuclei. which have more or less completely disappeared, is represented bv round or oval empty spaces. In thin, properly stained sections, this apparently solid protoplasmic mass is seen to possess a fibrillar structure. The fibres are largest and most dense around the nuclear spaces, and it seems reasonable to suppose that the appearance is due to a massing together of the various fibres into what appears to be a deeply staining membrane around the nuclear space such as would naturally be formed when the cells are crowded together. This picture is emphasized by rapidity of growth, and the fibrillar structure is lost except in the basement cells where the edges present a distinct brush-like border to the corium. Not only under the conditions which have just been mentioned is there proof of the unity of fibrils, but in the skin of the elephant previously described, a picture is presented (Fig. 2) suggesting unity of fibrils in the absence both of the separation of cells noted in the first description and the marked compression incident to the processes last described. Here the epidermis is in a state of normal equilibrium. There is not the irregularity incident to rapid proliferation and overgrowth. Consequently, transitional forms of these fibrils are not so numerous as in the more unstable situations. Nevertheless, the unity of the two forms of fibrils is more clearly suggested than in the human epidermis when under normal conditions fibrils are found only in the stratum filamentosum.

The detailed consideration of the relation of these fibrils to malignant growths of epithelial origin has been omitted, as not enough material was at hand to make a complete study of them. The greatest development of these fibrils, however, was seen in a rapidly growing squamous cell carcinoma of the tonsil. In this tumor there was the type of epithelial proliferation usually associated with this form of growth. Most of the mass consisted of irregular strands and nests of epithelial cells with very little connective-tissue stroma between the cell masses. These cell masses consisted of an outer zone of columnar cells, a mesial zone of polygonal cells, and a centre of flattened (in places cornified) epithelial cells. Throughout the section fibrils could be seen extending through certain cells of the outer zone and extending into and connecting the cells of the mesial zone. These fibrils differed in no way from the fibrils previously described. Arising apparently in the columnar cells, they serve to join together long rows of cells corresponding to those of the stratum filamentosum of normal epidermis. The irregular cell development of the tumor gave bizarre arrangements of fibrils, so that exaggerated reproductions of the process seen in Fig. 5 are apparent in discrete areas throughout the section. This reproduction of fibrils in squamous cell carcinoma, whereby the finer mechanism of epithelial-cell growth can be observed, would leave no doubt of the origin of these tumors if the point should be in question. It seems possible, therefore, that a further study of these fibrils may show them to be of importance in the identification or the grouping of tumors derived from squamous epithelium, since, where growth is most rapid, fibrils are most prominent.

From a general consideration of these specimens it is seen that in all sections of skin certain fibrils are present, which serve to bind together the cells of the epidermis, and are best seen in the stratum filamentosum where the cells are larger and paler and somewhat separated by lymph channels. These fibrils in normal skin are comparatively uniform and arranged with considerable regularity. In conditions of epithelial new growth these fibrils lose their regularity and uniformity, extending in places through several cells and branching off in various directions. Their increased prominence seems to be in direct ratio to rapidity of cell production. They can be traced in such conditions to fibrils in the stratum germinativum, with which they are in many instances directly continuous. These basement fibrils, which are practically wanting in normal human epidermis, are present in larger numbers in rapidly proliferating epidermis and are seen in many of the lower vertebrates.

Possibly the most important deduction to be drawn from the foregoing study of epidermal fibre production is that it establishes a relationship between the epidermal fibre system and the fibre system of other tissues, namely nervous, muscular, and connective-tissue structures.

We are now familiar with the fibrils of neuroglia cells, thanks especially to the work of Weigert and of Mallory; these cells are embryologically at least related to the cells of the epidermis, since both are of ectodermal origin. It has been shown by Southard[18] that in certain inflammatory processes of the cerebellum there is a change in the neuroglia cells, "such that numerous fibrils are thrown out which are roughly proportional to the number of cells present normally in the reacting area."

Recently, Mallory[19] discovered in connective tissue a hitherto undescribed fibril which he named the fibroglia fibril. These fibrils are produced by connective-tissue cells and are to be found normally in certain situations, but are most abundant in rapidly growing connective tissue, such as granulation tissue and the connective-tissue stroma of rapidly proliferating carcinoma. I have noted that those granulating ulcers of the skin that show the most marked production of epidermal fibrils also show, in the connective tissue below, large numbers of fibroglia fibrils.

It has been shown by Heidenhain[20] that muscle cells possess, in addition to their internal fibrils, certain peripheral fibrils which have been termed myoglia fibrils. These fibrils are, however, remarkably sensitive to post-mortem change, and it is doubtful if their full significance is yet understood. Quite recently fibrils in epithelial cells have been described by Wolbach.[21] He found in a carcinoma of the bladder fibrils varying in diameter, sometimes one and sometimes several to a cell, running longitudinally in the protoplasm of the cell.

## CONCLUSIONS.

In certain animals, in addition to the protoplasmic fibrils of the stratum filamentosum that are present normally in human epidermis, there are well developed fibrils in the cells of the stratum germinativum. These fibrils are present in the human epidermis only under conditions of increased cell activity.

These fibrils, that are prominent in the stratum germinativum of human epidermis under conditions of increased cell activity, seem to increase in direct ratio to rapidity of cell production.

The differentiation of these fibrils of the stratum germinativum into the finer fibrils which connect the cells of the stratum filamentosum can be observed under proper conditions of growth and therefore there should be no separation of the fibrils into different groups with various names.

This process of fibre production by the cells of the human epidermis is analogous to increased fibre production by various other cells under similar conditions, and may be of importance in identifying or classifying new growths of epithelial origin.

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## EXPLANATION OF PLATE XXIII.

Fig. 1.—From epidermis of zebra. Well developed fibrils are present both within and at the edges of the basement cells; some are of spiral form. Cells of the second layer are compact and joined by closely set protoplasmic fibrils, the ends of which are lost in the protoplasm of the cells.

Fig. 2.—From epidermis of the elephant. Perpendicular fibrils of the basement cell layer are prominent. The fibrils of the second layer are long, and irregular, reach the nucleus in places, and show distinct Ranvier-Bizzozero nodules.

Fig. 3.—Protoplasmic fibrils in basement cells of Necturus.

Fig. 4.—Œdema of skin with some proliferation of epithelium. A few of the long fibrils appear in the basement cells. Finer fibrils join all the cells (stratum germinativum and stratum spinosum). Ranvier-Bizzozero nodules are prominent.

Fig. 5.—Rapidly growing epidermis adjacent to ulcerating edge of carcinoma of lip. There is rich fibril production and the development of finer fibrils, connecting the cells of the mesial zone, from the long coarse fibrils of the basement cells is apparent. There are no Ranvier-Bizzozero nodules.

Fig. 6.—Papilloma of skin.

Fig. 7.—Palm of laborer showing excessive production of fibres.

Fig. 1

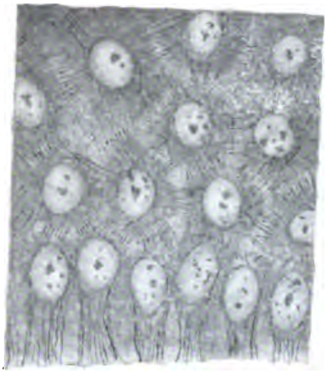


Fig. 2

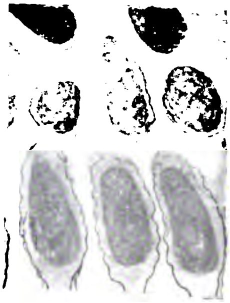


Fig. 3

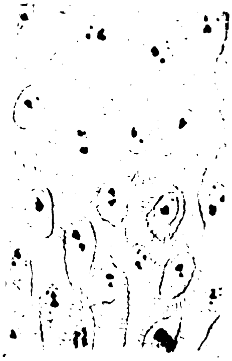


Fig. 4

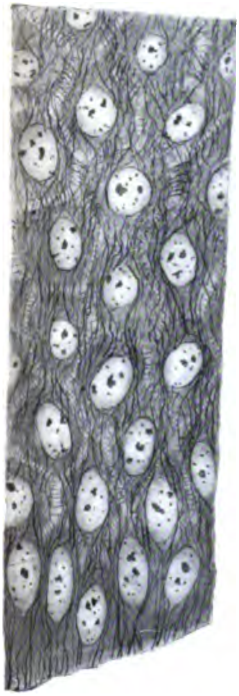


Fig. 6

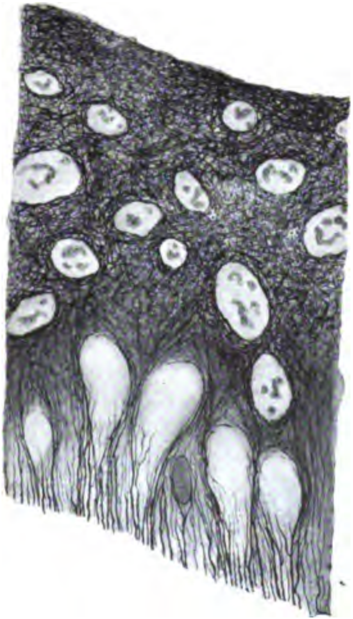
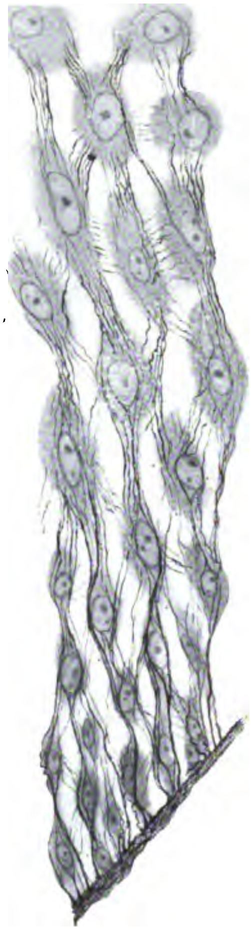


Fig. 7



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# ON THE INFLUENCE EXERTED ON THE VIRULENCE OF CARCINOMA IN MICE BY SUBJECTING THE TUMOR MATERIALS TO INCUBATION PREVIOUS TO INOCULATION.

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It is proposed to present in this communication the results of experiments made with a view to influencing the virulence of tumor cells by incubation previous to inoculation. The Jensen mouse carcinoma has been employed exclusively in this series of experiments, and, in view of the extensive literature on the subject, there is no need of further description of the tumor.

Jensen,[1] Loeb, Sticker, and Haaland have all, in the course of their transplantation experiments with animal tumors, determined the point at which the tumor materials, through exposure to high temperature, previous to inoculation, were rendered incapable of further proliferation. Jensen, working with his mouse carcinoma, found that exposure of the tumor materials suspended in normal salt solution (0.8 per cent.) to temperatures of 47° C. and upwards for a period of five minutes entirely destroyed the tumor cells, whilst at a temperature of 46° C. a distinct diminution in the yield of tumors, as compared with that obtained at lower temperatures, was observed. The number of animals employed in this experiment was extremely small, no control was made at normal temperatures, and no statements were made regarding the relative speed at which individual tumors in the different series developed.

Loeb[2] observed that exposure of the rat sarcoma, with which he was working, to temperatures of 45° C. and upwards for periods of thirty minutes or more, was sufficient to prevent further development on transplantation. In one case, employing materials which had been incubated forty minutes at 43° C., a fairly good yield was obtained (four fatal tumors in seven rats). He found, however, in those cases in which temperatures approaching 43° and 44° C. had been used, that the tumors made their appearance later than in the control series and that their speed of development was markedly retarded.

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[1] Jensen, *Centralblatt f. Bakteriologie*, Abt. I, Orig., 1903, xxxiv, 122.

[2] Loeb, *Virchow's Archiv*, 1903, clxxii, 345.

Sticker,[3] in an extensive series of experiments on the transplantation of a lymphosarcoma in dogs, made a single experiment in which small pieces of the tumor were subjected to a temperature of 50° C. for two hours previous to their introduction subcutaneously into a two-months-old puppy. Unfortunately the dog died of pneumonia ten days after the commencement of the experiment, but examination showed tumors to be developing at both points of inoculation, indicating that lymphosarcoma of this type was capable of resisting much higher temperatures than were the materials employed in the experiments recorded above.

Haaland,[4] in a recent communication from Ehrlich's laboratory, published the results of a series of experiments carried out on a mixed mouse tumor with a view to obtaining a sarcoma free from carcinoma cells. The tumor materials were introduced into thin-walled capillary tubes which were heated in a water-bath at a temperature of 44° C., for periods of five, ten, and fifteen, up to sixty-five minutes respectively. In a control series of six mice which had received normal materials without previous incubation, 80 per cent. yielded rapidly-growing tumors, whilst in the heated series the yield of tumors and the speed of development both diminished with the increasing period for which the materials had been incubated previous to inoculation. It was also observed that the carcinoma cells succumbed earlier than the sarcoma cells, with the result that materials which had been treated for thirty-five minutes and upwards yielded pure sarcomata in which no trace of carcinoma was to be found. Haaland considered that the reduction in virulence and speed of development was not attributable solely to the destruction of a large proportion of the cells, but rather to some deep-seated change in the cell mechanism, indicated by the histological findings.

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[3] Sticker, *Zeitschrift f. Krebsforschung*, 1904, i, 413.

[4] Haaland, *Berl. klin. Wochenschr.*, 1906, xliii, 40.

It will be observed that all these authors made use of relatively high temperatures and for the most part of fairly virulent materials, confining their attention principally to a determination of the point at which the cells employed were no longer capable of proliferation. From the statements of Loeb and Haaland, it is apparent that not only was the number of tumors in parallel series decreased with increasing temperatures to which the materials had been exposed previous to inoculation, but, further, a distinct retardation in the development of individual tumors of the high-temperature series was noted by both observers.

All those who have carried on extensive experiments on the transplantation of animal tumors are aware of the extraordinary variations which occur in one and the same series, both in the percentage of tumors and in the speed of development of individual tumors of the series, all of which might be attributable to a variation in the concentration of a stimulating agent causing proliferation of the cancer cells.

Our experiments started with the conception that exposure to temperatures slightly above the normal for short periods of time might exert an effect upon weak and attenuated tumor cells, stimulating them to more active development on subsequent inoculation in a manner analogous to the action of poisons at high dilution on yeasts, bacteria, etc. The activity of catalytic chemical agents on enzymes, toxins, etc., has been demonstrated to increase with increasing temperatures, and since the stimulating effect of poisons is exerted only within very narrow limits of concentration, it is quite conceivable that a slight rise in temperature might so increase the activity of a stimulating body already present as to lead to a marked increase in the proliferative energy of the cell.

Before commencing the series of experiments in which it was necessary to make relatively accurate comparisons between the results obtained in experimental series and their controls, it was necessary to eliminate as far as possible all sources of experimental error calculated to introduce accidental variations. Our general procedure when inoculating mice, both in these and other experimental series, was as follows: a mouse having a suitable tumor was killed, the tumor removed and weighed and ground in a sterile mortar with three or four times its volume of salt solution. All coarse particles which might interfere with the uniformity of the mixture were removed by means of rakes and in some cases by filtration through coarse gauze, a procedure which, whilst calculated to afford a perfectly homogeneous mixture for experimental work, would also tend to reduce the percentage of tumors. The contents of the mortar were then measured and divided into three or four equal parts in sterilized test tubes. One portion was reserved for purposes of control, the remaining tubes being subjected to the required temperatures for a definite period of time in the Ostwald water-bath. After the completion of the incubation process, the contents of each test tube were introduced into a fresh sterile mortar, once more thoroughly admixed, and uniform doses, usually from 0.4 to 0.5 cubic centimeter of the mixture injected subcutaneously into each mouse of the series. The greatest care was observed in carrying out the series of experiments that the mice selected were all derived from the same source and approximately the same age, so that, employing for example, ten mice for the normal materials and ten each for those which had been incubated at each temperature, it might be said that the only varying factor introduced into these experiments was the influence exerted on the cancer material by subjecting it to incubation previous to inoculation.[5]

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[5] As a control on the incubation experiments in this paper, the results obtained by inoculating two or more lots of mice on the same day with the same normal materials are of value. Without observing as extensive precautions as in the experimental series, the yields obtained in various comparable groups were so nearly uniform as to make it extremely probable that the results observed in the incubated series were attributable to the experimental treatment and not to accidental distribution. For example, lots 7 $\alpha$ and 7 $\beta$, inoculated June 10, 1904, with the same material, yielded, respectively, 4 tumors in 13 mice and 4 tumors

The mere enumeration of the tumors obtained would of course afford but a very inadequate idea of the relative virulence of tumor materials employed for the inoculation of comparative series. We were led, therefore, early in the course of our experimental work to adopt a system of identifying individual members of a group and following the development of their tumors at short intervals of time from inoculation to death. For this purpose chart books were employed in which the groups were arranged numerically and the individual members of a group alphabetically. For each individual mouse a series of diagrams was prepared showing the fore legs, hind legs, nose, and tail. When a tumor made its appearance, it was reproduced in the location in which it occurred, in a diagram as nearly life size as possible, and as the tumor increased in bulk and secondary tumors made their appearance, further chartings were prepared showing the progress of the growth until the death of the animal occurred or spontaneous recovery intervened. (Gaylord and Clowes, On Spontaneous Cure of Cancer, *Surgery, Gynæcology, and Obstetrics*, 1906, ii, 633.)

Making use of such a system, it was possible to determine not only the actual number of fatal tumors in each group, but also the relative speed of development of individual tumors in the series, as indicated by the number of days from inoculation required to reach a certain size, and the volume of the tumors after a given number of days.

It would of course be preferable in a publication of this nature to include the complete charted records of every series of experiments, but since, on account of space limitations, this is impossible, reference has been made to the records wherever necessary in order to bring out the essential points.

The greatest variation in the virulence of tumors is frequently observed in the same series. The age of a tumor, that is to say,

in 11 mice. Lots 161 and 162 each of ten mice, inoculated Dec. 21, gave 3 large tumors apiece. In other cases a difference of one or at most two tumors is observed, but in no normal cases are the differences in the yields as great as those recorded in the incubated series dealt with subsequently in this paper, in spite of the fact that practically no precautions were observed to insure uniformity.

the period which has elapsed between the date of inoculation and the date on which the tumor has attained a size suitable for transplantation, frequently bears a marked relation to the yield of tumors obtained in the following generation. An illustration of this form of variation is to be found in the following table in which are compared the yields obtained on further transplantation from a series of six tumors, all of which belonged to the same group, having been inoculated on the same day with the same materials.

| Tumor | Age in days | No. mice employed | No. of tumors | Percentage of tumors. |
|---|---|---|---|---|
| 214 A | 45 | 19 | 8 | 42.0 |
| 216 B | 56 | 7 | 2 | 28.5 |
| 213 B | 59 | 38 | 11 | 29.0 |
| 214 B | 66 | 36 | 10 | 28.0 |
| Total 66 days and under | | 100 | 31 | 31.0 |
| 218 E | 184 | 26 | 5 | 19.2 |
| 215 B | 184 | 32 | 2 | 6.0 |
| Total 184 days | | 58 | 7 | 12.0 |

It will be seen that the yield has fallen from 31 per cent. in the rapidly growing tumors to 12 per cent. in those which required upwards of six months for development.

It has also been noted that very young tumors weighing less than four grams seldom give good results on transplantation. In fact, the best yields are obtained, as a rule, from tumors that are still developing fairly actively and weigh over five grams.

After preliminary experiments from which it was concluded, that no tumors could be obtained from materials incubated at temperatures above 44° C. for thirty minutes or more, we commenced the following series of experiments.

Experiment I.[6] November 19, 1904.

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[6] In order to avoid confusion, we have tabulated the results as far as possible. Under the lot numbers of comparable experimental series are found first the number of mice employed, followed by the treatment to which the tumor materials had been submitted previous to inoculation, and the results of examination of such series after a period of 8 months.

Mouse tumor 105 C employed for inoculation. Weight of mouse, 18.5 grams. Weight of tumor, 6.5 grams.

Age of tumor (time elapsed from date of inoculation to Nov. 19), 40 days.

Ratio of tumor to salt solution, 1:4. Dose, 0.5 c.c.

| Serial No. | Lot 142 | Lot 143 | Lot 144 |
|---|---|---|---|
| Number of mice | 8 | 8 | 8 |
| Incubation of tumor materials previous to inoculation | Nil | 30 mins. at 40°C. | 30 mins. at 45°C. |
| Examination after 3 mos. | 6 large tumors | 1 large tumor | Nil |

In the above series of experiments the tumor employed for inoculation was derived from an extremely virulent strain and incubation at 40° C. was found to lower the yield of tumors from 75 to 12.5 per cent.[7]

Experiment II. December 5, 1904.

Mouse tumor 101 D employed. Weight of mouse 22.5 grams. Weight of tumor, 3.5 grams.

Age of tumor, inoculation to death, 66 days.

First appearance of tumor to death, 28 days.

Ratio of tumor to salt solution, 1:4. Dose, 0.5 c.c.

| Serial No. | Lot 119 | Lot 150 | Lot 151 |
|---|---|---|---|
| Number of mice | 8 | 8 | 8 |
| Incubation of tum. mat. previous to inoculation | Nil | 10 min. at 35° C. | 10 min. at 40° C. |
| Examination after 3 mos. | 4 large tumors | 2 tumors<br>1 spon. recovery | 1 large tumor |

The tumor employed in this experiment was also derived from a virulent strain and although small it developed rapidly in the course of the few days preceding date on which the animal was killed. It was moderately necrotic and from its appearance was expected to yield a fair proportion of tumors. As has been seen from the table, the yield decreased with increasing temperatures to which the materials were submitted previous to inoculation.

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[7] The percentage terms used in this case are simply for purposes of comparison, as it would obviously be impossible to make any calculations based upon such a small number of animals.

Experiment III. March 17, 1905.

Mouse tumor 251 E employed. Origin, 142–105.[8] Weight of mouse, 52.5 grams. Weight of tumor 6.2 grams.

Age of tumor, inoculation to death, 53 days.

From first appearance of tumor to death, 36 days.

Ratio of tumor to salt solution, 1:4. Dose, 0.5 c.c.

| Serial No. | Lot 166 | Lot 174 | Lot 190 |
|---|---|---|---|
| Number of mice | 9 | 9 | 6 |
| Incub. of tum. mat. previous to inoculation | Nil | 15 min. at 40° C. | 15 min. at 45° C. |
| Period after inoculation. | Record of results. | | |
| 10 days. | 2 small tum. | 6 small tum. | Nil |
| 15 days. | 2 of 5 mm. diam.<br>2 of 3 mm. diam. | 3 of 10 mm. diam.<br>3 of 6 mm. diam. | Nil |
| 31 days. | 2 of 10 mm. diam.<br>2 of 5 mm. diam. | 1 death of tum.<br>4 lg. 1 sm. tum. | Nil |
| Final results, 4 months. | 3 large tum.<br>1 small tum. | 5 large tum.<br>1 small tum. | Nil |

As a final result it will be seen that five large tumors and one small tumor occurred in the incubated series in comparison with three large and one small in the normal series. In addition, it should be noted that the speed of development of the tumors in Lot 174 was far greater than that of the tumors of Lot 166. Fifteen days after inoculation the former were as far advanced as were the latter at the end of 31 days. Lot 174 afforded us a remarkable illustration of extremely rapidly developing tumors which became necrotic and were suitable for transplantation at a much earlier date than was normally found to be the case.

Experiment IV. April 7, 1905.

Mouse tumor 198 B employed. Origin 150 B–101. Weight of mouse 27.4 grams. Weight of tumor, 6.6 grams.

Age of tumor, inoculation to death, 38 days.

First appearance of tumor to death, 27 days.

Ratio of tumor to salt solution, 1:3.

Materials filtered through gauze and consequently considerably attenuated. Dose, 0.4 c.c.

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[8] In this and subsequent cases in which the origin of the tumor is given its direct descent is traced to one of the four groups, 101, 102, 105, 106, all of which were in the second generation from the original Jensen tumor. For family tree showing relationship of individual groups see *American Journal of Physiology*, 1905, xiv, 177.

| Serial No. | Lot 227 | Lot 224 | Lot 225 | Lot 226 |
|---|---|---|---|---|
| Number of mice | 6 | 6 | 6 | 6 |
| Incubation | Nil | 30 min. at 40° C. | 2 hr. at 40° C. | 19 hr. at 40° C. |
| Examination after 4 Mos. | No tumors | 1 tumor | 1 tumor | No tumors |

The low yield of tumors in this series is probably attributable in part to the filtration through gauze but it should also be noted that the tumor in question was young and in active development and showed very limited areas of necrosis.

Experiment V. April 19, 1905.

Mouse tumor 41 B employed. Origin of tumor, 147–105. Weight of mouse 30.5 grams. Weight of tumor, 5.2 grams.

Age of tumor, inoculation to death, 62 days.

First appearance of tumor to death, 40 days.

Ratio of tumor to salt solution, 1:4.

Material filtered through gauze. Dose, 0.4 c.c.

| Serial No. | Lot 234 | Lot 235 |
|---|---|---|
| Number of mice | 6 | 6 |
| Incubation | Nil | 1 hour at 41° C. |
| Examination after 6 mos. | 1 slow-growing tumor weighing 1 to 2 grams | 4 large rapidly growing tumors weighing 5 to 9 grams. |

Experiment VI. April 19, 1905.

Mouse tumor 199 F employed. Origin, 150 B–101. Weight of mouse, 24.2 grams. Weight of tumor, 9.3 grams.

Age of tumor, inoculation to death, 50 days.

First appearance of tumor to death, 38 days.

Ratio of tumor to salt solution, 1:4.

Material filtered through gauze. Dose, 0.4 c.c.

| Serial No. | Lot 236 | Lot 237 |
|---|---|---|
| Number of mice | 7 | 6 |
| Incubation | Nil | 1 hour at 41° C. |
| Examination after 4 mos. | 1 slow-growing tumor | 1 rapidly growing tumor. |

The extreme attenuation in this group resulting in the production of one tumor only in each of the series is probably attributable to the filtration through gauze.

The four preceding series are remarkable in that in no case is there found to be a smaller yield of tumors in the groups incubated at 40° or 41° C. than in those which received no treatment. Summarizing the results it may be said that whilst six slow-growing tumors were obtained from thirty mice in the normal series, ten rapidly growing and two slow-growing tumors, making a total of twelve tumors, were obtained from twenty-nine mice in the groups incubated at 40° or 41° C. If we exclude the two series in which filtration through gauze has led to such a marked attenuation that four tumors only were obtained from the inoculation of thirty-nine mice, we find that in the two remaining series five slow-growing tumors were obtained from fifteen mice in the normal groups, as against eight fast- and one slow-growing tumors out of fifteen mice in those groups which had received injections of tumor materials previously incubated at 40° or 41° C.

Having thus demonstrated that incubation at relatively high temperatures is not necessarily prejudicial, and, in the case of those tumors which afforded a small yield at normal temperatures, actually led to an increase, we determined to employ tumors known to possess a high degree of virulence. For this purpose two tumors of Lot 174 were chosen for the following experiments, VII and VIII. This group, referred to above in Experiment III, was characterized by the extreme rapidity with which the tumors of its members reached maturity, as indicated by a peculiar type of non-infected necrosis, which has been found on experiment to yield the maximum of tumors on transplantation.

Experiment VII. May 5, 1905.

Mouse tumor 174 G employed. Origin, 251 E–142–105. Weight of mouse, 22.0 grams. Weight of tumor, 5.2 grams.

Age of tumor, inoculation to death, 49 days.

First appearance of tumor to death, 35 days.

Tumor partially necrotic but not infected.

Ratio of tumor to salt solution, 1:4. Dose, 0.5 c.c.

| Serial No. | 321 | 322 | 323 | 324 |
|---|---|---|---|---|
| Number of mice | 8 | 8 | 8 | 8 |
| Incub. of tum. mat. previous to inoc. | Nil | 1 hr. at 40° C. | 1 hr. at 42°C. | 1 hr. at 44°C. |
| Period after inoculation | | Record of results | | |
| 24 days. | 4 large tum.<br>2 small tum. | 1 large tum.<br>3 small tum. | Nil | 1 small tum. |
| Final results after 4 mos. | 6 large tum.<br>1 intermed. t. | 3 large tum.<br>1 small tum. | 1 small tum. | 1 large tum. |

It will thus be seen, to summarize the results obtained by making use of this extremely virulent material, that employing eight mice in each case, seven tumors were obtained in the normal series, four in the series incubated at 40° C., and one each in the series incubated at 42° and 44° C.

Experiment VIII. May 16, 1905.

Mouse tumor 174 B employed. Origin, 251 E–142–105. Weight of mouse 24.8 grams. Weight of tumor, 4.3 grams.

Age of tumor, inoculation to death, 60 days.

First appearance of tumor to death, 50 days.

Tumor partially necrotic but not infected.

Ratio of tumor to salt solution, 1 : 4.5. Dose, 0.5 c.c.

| Serial No. | 329 | 330 | 331 | 332 |
|---|---|---|---|---|
| Number of mice | 8 | 8 | 8 | 8 |
| Incub. of tum. mat. previous to inoc. | Nil | 1 hr. at 39° C. | 1 hr. at 41° C. | 1 hr. at 43° C. |

| Period after inoc. | Record of Results | | | |
|---|---|---|---|---|
| 28 days | 1 large, 2 mod. and 1 small tum. | 1 mod. tumor | 1 mod. tumor | Nil |
| Final results, 4 months | 5 large tumors | 3 large tumors | 2 large tumors | Nil |

To summarize the results it may be said that there developed in the normal series five tumors, in the series incubated at 39° C. three tumors, in that at 41° C. two tumors, and at 43° C. nil.

In the two preceding series, VII and VIII, we were dealing, as in Experiments I and II, with materials above the normal average of virulence and in both cases incubation appears to diminish the proportion of successful transplantations, and, further, to effect a slight retardation in the speed of development as compared with the normal.

On May 19 and 23, two series of experiments were carried out in which the materials were incubated two hours at 43° to 44° C. previous to inoculation. This treatment produced such a marked inhibition, only one tumor being obtained in the entire series, that we have excluded the experiment in question and must assume that such a prolonged treatment at a temperature as high as 44° C. is capable of entirely destroying the tumor cells, thus rendering further proliferation impossible.

An analysis of the results obtained in the preceding eight series made it appear probable that whilst treatment at a temperature of 38° to 41° C. was distinctly unfavorable to the development of virulent tumors, the same process produced a stimulating effect leading to an increased proportion of tumors as compared with the normal when non-virulent materials were employed.

In the six remaining series an attempt was made to compare the results of slowly and rapidly growing tumors, of small and large tumors, etc.

Experiment IX. October 11, 1905.
Mouse tumor 340 A employed. Origin, 213 B-175-161-128-101. Weight of mouse, 32.75 grams. Weight of tumor, 6.4 grams.
Age of tumor, inoculation to death, 138 days.
First appearance of tumor to death, 119 days.
Tumor only slightly necrotic.
Ratio of tumor to salt solution, 1:3. Dose, 0.6 c.c.

| Serial No. | 469 | 470 | 471 |
|---|---|---|---|
| Number of mice | 8 | 8 | 8 |
| Incub. of tum. mat. previous to inoc. | Nil | 30 min. at 38.5° C. | 30 min. at 42.2° C. |
| Period after inoc. | | Record of results | |
| 41 days | 1 mod. tumor | 2 mod. tumors | |
| | 1 small tumor | 2 small tumors | 2 small tumors |
| 58 days | 1 mod. tumor | 2 large tumors | |
| | 1 small tumor | 2 small tumors | 1 small tumor |
| Final results, | 2 mod. tumors | 2 large tumors | |
| 4½ months | | 1 small tumor | Nil |

Experiment X. October 11, 1905.
Mouse tumor 427 A employed. Origin, 395 C-232 B-195 D-169 C-106. Weight of mouse, 37.9 grams. Weight of tumor, 9.9 grams.
Age of tumor, inoculation to death, 43 days.
First appearance of tumor to death, 30 days.
Tumor far more necrotic than 340 A used in Experiment IX.
Ratio of tumor to salt solution, 1:3. Dose, 0.7 c.c.

| Serial No. | 472 | 473 | 474 |
|---|---|---|---|
| Number of mice | 10 | 10 | 10 |
| Incub. of tum. mat. previous to inoc. | Nil | 30 min. at 38.5° C. | 30 min. at 42.2° C. |
| Period after inoc. | | Record of results | |
| 41 days | 4 mod. tumors | 2 mod. tumors | Nil |
| | 2 small tumors | 3 small tumors | |
| 58 days | 4 large tumors | 1 large tumor | Nil |
| | 2 mod. tumors | 1 mod. tumor | |
| Final Results, | 6 large tumors | 1 large tumor | Nil |
| 4½ months | | 1 mod. tumor | |

The above results obtained in Experiments IX and X are interesting in view of the fact that they afford us a comparison between two tumors which showed a great difference in their speed of development. 340 A, used in Experiment IX, was inoculated on May 26, showed a tumor as early as June 14, which increased in size until June 26, on which date it diminished and remained stationary for a couple of months, finally about the beginning of September developing once more until on October 11 it had reached a sufficient size to be used for transplantation.

Mouse No. 427 A, used in Experiment X, was inoculated August 29 and its tumor had reached considerable dimensions by September 18 and as stated above weighed almost 10 grams on October 11. Thus the tumor which required 138 days to reach 6.4 grams showed practically uniform results in the three series of experiments, an actual increase in the number of tumors being noted at a temperature of 38.5° C., whilst the tumor which required only forty-three days to reach a weight of 9.9 grams and showed marked necrosis, gave a large yield, 66 per cent., at normal temperatures, falling to 22 per cent. at 38.5° C., and to nothing at 42° C.

Experience had shown that small, hard tumors gave extremely poor yields on transplantation. We determined therefore to make use of two tumors, both of which had been inoculated on the same day, one of which had grown slowly and the other rapidly.

Experiment XI. October 25, 1904.

Mouse tumor 429 A employed. Origin, 328 C–214 A–175 C–161 M–128–101. Weight of mouse, 24.7 grams. Weight of tumor, 4.2 grams.

Age of tumor, inoculation to death, 48 days.

First appearance to death, 30 days.

Tumor small, hard, and not necrotic.

Ratio of tumor to salt solution, 1:3. Dose, 0.5 c.c.

| Serial No. | 505 | 506 | 507 |
|---|---|---|---|
| Number of mice | 10 | 10 | 10 |
| Incub. of tum. mat. previous to inoc. | Nil | 20 min. at 36° C. | 20 min. at 40.5° C. |
| Period after inoc. | | Record of results | |
| 27 days | 3 small tumors | 2 mod. tumors<br>4 small tumors | 1 large, 1 mod., and 5 small tum. |
| 44 days | 2 small tumors | 3 large tumors<br>3 med. tumors | 4 large, 1 mod., and 2 small tum. |
| Final results, 4 months | 1 small tumor | 5 large tumors<br>1 med. tumor | 4 large tumors<br>1 med. tumor |

Experiment XII. October 25, 1904.

Mouse tumor 432 B employed. Origin, 329 E–174 B–100 E–142–105. Weight of mouse, 36.2 grams. Weight of tumor, 11.8 grams.

Age of tumor, inoculation to death, 48 days.

First appearance of tumor to death, 25 days.

Tumor large, soft, cystic, and necrotic but not infected.
Ratio of tumor to salt solution, 1:3. Dose, 0.5 c.c.

| Serial No. | 508 | 509 | 510 |
|---|---|---|---|
| Number of mice | 10 | 10 | 10 |
| Incub. of tum. mat. previous to inoc. | Nil | 20 min. at 36° C. | 20 min. at 40.5° C. |
| Period after inoc. | | Record of results | |
| 27 days | 2 med. tumors<br>1 small tumor | 1 med. tumor<br>2 small tumors | 1 med. tumor<br>1 small tumor |
| 44 days | 2 large tumors<br>2 med. tumors | 1 large tumor<br>3 med. tumors | 1 large tumor |
| Final results after 4 months | 2 large tumors<br>2 med. tumors | 4 large tumors | 1 large tumor |

Comparing the results of the two preceding experiments it will be seen that whilst the incubation of the small, hard tumor led to a great increase, from 10 to 60 per cent., in the yield of tumors in the succeeding generation, the incubation of the large, soft, cystic tumor produced relatively less effect, a decrease in the proportion of tumors at 40° C. being noted, as compared with the normal.

The final series of incubation experiments was carried out making use of two slow-growing tumors both of which had shown at some stage more or less tendency to remain stationary or retrogress.

Experiment XIII. December 12, 1904.
Mouse tumor 419 B employed. Origin, 383 A–304 A–200 A–150 B–101.
Weight of mouse, 18.8 grams. Weight of tumor, 2.9 grams.
Age of tumor, inoculation to death, 112 days.
First appearance of tumor to death, 80 days.
Ratio of tumor to salt solution, 1:4. Dose, 0.4 c.c.

| Serial No. | 590 | 591 | 592 |
|---|---|---|---|
| Number of mice | 8 | 8 | 8 |
| Incub. of tum. mat. previous to inoc. | Nil | 30 min. at 37° C. | 30 min. at 40° C |
| Period after inoc. | | Record of results | |
| Final results after 10 weeks | 2 large tumors | 4 large tumors<br>1 mod. tumor | 1 large tumor<br>1 small tumor |

Experiment XIV. December 12, 1904.
Mouse tumor 462 C employed. Origin, 218 E–175 C–161 M–128–101
Weight of mouse, 33.6 grams. Weight of tumor, 5.3 grams.
Age of tumor, inoculation to death, 74 days.
First appearance of tumor to death, 40 days.
Ratio of tumor to salt solution, 1:3. Dose, 0.5 c.c.

| Serial No. | 593 | 594 | 595 |
|---|---|---|---|
| Number of mice | 10 | 9 | 10 |
| Incub. of tum. mat. previous to inoc. | Nil | 30 min. at 37° C. | 30 min. at 40° C. |
| Period after inoc. |  | Record of results |  |
| Final results after 10 weeks | 2 large tumors | Nil | 4 large tumors |

These two series show, as would be expected, a rather larger proportion of tumors in the incubated than in the normal series; whilst 419 B shows a maximum at 37° C., 462 C gives the highest yield from materials incubated at 40° C. The complete absence of tumors in lot 594 is extremely difficult to explain, and forms the most serious exception in the entire series of experiments enumerated above. We have endeavored to find some explanation for this discrepancy. The ulceration of tumor materials in some mice of this series would indicate a possible contamination during the course of the incubation process. We are not however aware of any such accident having occurred, and must therefore conclude that in spite of the utmost precautions, discrepancies will occur when dealing with materials of this nature.

Excluding the two series of experiments in which filtration through gauze or exposure to temperatures above 44° C. for more than an hour would materially interfere with results, we find that twelve series of experiments have been carried out employing thirty-seven lots of mice, each of which contained from six to ten individuals.

We have included two tables in which the results obtained after three months by incubating five strong and five weak strains at 40° to 41° C. are collected. In the first column is given the number of the mouse employed originally for inoculation of the series. In the second column is the number of mice employed in each series both for the normal and incubated material. In the third column is found the number of tumors present in the normal series, the number preceding the + sign representing the rapidly growing tumors and that following, the slowly growing tumors. In the final column is found the number of rapidly and slowly growing tumors in the incubated series.

TABLE I. VIRULENT GROUPS.

| Mouse | Number of mice in each series | Tumors of normal series | Tumors of incubated series |
|---|---|---|---|
| 105 C | 8 | 6 + 0 | 1 + 0 |
| 101 D | 8 | 4 + 0 | 1 + 0 |
| 174 G | 8 | 6 + 1 | 3 + 1 |
| 174 B | 8 | 4 + 1 | 1 + 1 |
| 432 B | 8 | 2 + 2 | 1 + 0 |
| Total | 40 | 22 + 4<br>55% + 10% = 65% | 7 + 2<br>17.5%+5%=22.5 % |

TABLE II. NON-VIRULENT GROUPS.

| Mouse | Number of mice in each series | Tumors of normal series | Tumors of incubated series |
|---|---|---|---|
| 251 | 9 | 3 + 1 | 5 + 1 |
| 41 B | 6 | 0 + 1 | 4 + 0 |
| 429 A | 7 | 0 + 1 | 4 + 1 |
| 419 B[9] | 8 | 2 + 0 | 1 + 1 |
| 462 C | 10 | 2 + 0 | 4 + 0 |
| Total | 40 | 7 + 3<br>17.5% + 7.5% = 25% | 18 + 3<br>45% + 7.5% = 52.5% |

In some cases the maximum is obtained at 37° or 38° C., a fall already being noticed at 40° and 41° C. Temperatures of 42° C. and upwards seem in all cases to exert an inhibiting effect upon the tumor development.

An inspection of the above table shows that by incubating the tumor materials in salt solution for varying periods of time at a temperature equal to the maximum fever temperature, a greatly increased yield was obtained from relatively weak strains, the total number of tumors being more than twice as great in the same number of mice. On the other hand, incubation at the same temperature produced a marked inhibiting effect where highly virulent materials were employed, a fall to less than one-third of the number of rapidly growing tumors and from 65 to 22.5 per cent. in the total yield being recorded.

Finally, reference should be made to practical application of incubation as a means of stimulating proliferation of tumors. The transplantation of a primary mouse carcinoma presents far greater difficulties than does the reinoculation of such a tumor in subsequent generations. Any means, therefore, of stimulating the cancer cells to more active proliferation must possess considerable value from an experimental standpoint. Several mice having primary carcinoma similar to that of the Jensen mouse have been sent to the laboratory from a common source in the course of a few months. All attempts which were made to transplant the carcinomatous tissue by ordinary means having failed, it was decided to make an attempt to increase the virulence of any subsequent tumor of this group by incubation previous to inoculation. This method afforded extremely satisfactory results, a considerable tumor being obtained in a mouse inoculated with materials which had previously been incubated twenty minutes at 39° C. This tumor was incubated and transplanted, and three tumors were obtained in the following generation. The tumor has now been transplanted through three generations and may therefore be looked upon as successfully established.

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[9] 419 B gave a maximum yield of 50 % on incubation at 37° C. and presumably was already suffering attenuation when heated to 41° C.

The most remarkable facts in connection with this experiment are:

1. Whilst 27 mice which have received incubated material have yielded 8 tumors, 15 mice which received identically similar materials, but without incubation previous to inoculation, have yielded no tumors.
2. The virulence of the tumor has increased in the course of three generations to a marked degree. Whilst ten weeks were required for the development of the first tumor, and five weeks for those in the second generation, tumors of considerable size have already made their appearance in the third generation in less than a week.

We do not attribute any special significance to a single experiment of this nature, but since it appears probable that incubation was instrumental in establishing this series, we are making use of incubation for half an hour at temperatures of 38.5° to 39.5° C. in all cases in which primary tumors are sent to the laboratory, and have included a note on the subject in the hope that our method may possibly prove of value to others working in the same field.

## SUMMARY AND CONCLUSIONS.

Our experience with mouse tumors has demonstrated that a great difference exists not only in the relative immunity possessed by different individuals but also in the relative virulence of different tumors all owing their origin to the same source.

In this paper it has been shown that the variations in question can be accentuated by incubation of the transplantation materials previous to inoculation at temperatures varying from 37° to 41° C. In the case of extremely virulent tumors giving a yield of upwards of 60 per cent. under normal conditions, a distinct and gradual fall in virulence accompanies increasing temperatures of incubation until a point is reached at which no further tumors are obtained. On the other hand, relatively inactive materials giving comparatively small yields of tumors under normal conditions may be so stimulated by subjecting them to incubation at suitable temperatures as to afford a larger yield and relatively more rapidly-growing tumors than were obtained in the normal series. In these cases the optimum temperature (that is to say, the temperature at which, when incubated for a definite period of time the materials employed yield the largest proportion of tumors) appears to vary, for whilst the most virulent materials, as stated above, exhibit an optimum yield at normal temperatures decreasing with incubation, less virulent materials show an optimum at temperatures slightly above the normal, whilst the least virulent of all show an optimum at as high a temperature as 40° to 41° C., all the curves eventually falling to zero, as those temperatures are reached at which the cells themselves might be expected to succumb.

This fact having been demonstrated, it remains to compare the histories of the various tumors employed for the sixteen series of experiments. The charted diagrams representing the course of development of each individual tumor from the date of inoculation to the date of death have been prepared and afford an admirable means of comparison of the relative speed of development of the tumors employed and consequently of their relative proliferative energy. The origin of all tumors for several generations, whether from a relatively active or inactive stock, may be determined from the records, and data regarding the age, weight, and pathological state of the tumor are also available.

1. Those tumors which give a small yield under normal conditions, but which give an increased yield on incubation, are either rapidly growing, young tumors, or small and hard tumors, or have been extremely slow in their development, and have in

certain cases exhibited indications of a tendency to undergo spontaneous recovery. Tumors of this class seldom exhibit any marked indications of necrosis of the type which will be referred to in the next paragraph.

2. Those tumors which on inoculation give a large yield at normal temperatures and a gradually decreasing percentage of tumors in the batches inoculated with materials which have been incubated at increasing temperatures are generally fully grown tumors which have developed rapidly and come of a relatively active strain. They are usually softer and more cystic than those considered in the previous section. Their most marked characteristic is, however, a state of necrosis accompanied by an increasing calcium content without an entire disappearance of potassium.[10] This state of necrosis is peculiar in that it is apparently not attributable to the intervention of bacteria, very few of which are present in the most satisfactory transplantation materials, but rather to some auto-digestive or enzymotic process at work within the cells.[11]

The enormous proliferative power possessed by the cells of this Jensen tumor has frequently been referred to by other workers who have employed the same materials. Such a remarkable development may most readily be explained as due to the intervention of some agent continually or intermittently stimulating the cells to further proliferation. We are not concerned at the present moment with the exact chemical composition of the body in question, as to whether it is possessed of an enzymotic or toxic nature or consists simply of a complex aggregate possessing a well-defined chemical composition. Neither are we attempting to determine the source of the body in question; whether it be derived from some parasite which has invaded the cell, or is itself a product of the impaired metabolism of the cancer cell. It is simply proposed to consider the probable action, at varying concentrations, of a chemical body capable of exerting a stimulating action on the cell which reaches an optimum at a given concentration and follows certain laws governing chemical action.

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[10] Clowes and Frisbie, *American Journal of Physiology*, 1905, xiv, 173.

[11] In all those cases in which tumors have been infected they have proved totally unfit for transplantation experiments, leading either to the death of the mouse or to an ulceration process whereby the infected materials were entirely removed, no tumors resulting from such a treatment.

Poisons which cause the death of an organism at concentrations exceeding a given limit are in all cases capable of producing a stimulating effect upon that same organism when employed at sufficient dilution, whilst below that optimum point they produce little or no effect of any kind. Thus, for example, mercuric chloride inhibits absolutely the development of yeast at a concentration of one part in 20,000, whilst at a dilution of 1 in 330,000 the proliferative power of the yeast subjected to its action is actually increased.[12] The same effect is exerted upon enzymes; diastase, for example, is stimulated in its activity by the addition of extremely small amounts of sulphurous acid, oxalic acid, etc., whilst had the concentration been ten to forty times as great, the action of the diastase would have been nil, or practically inhibited.[13] It should be noted under this head that the stimulating effect of such toxic bodies is exerted only within extremely narrow limits, usually ranging from concentrations about five times as great to concentrations about five times as dilute as the optimum.

A second law which is applicable to the case in point is that governing the action of chemical bodies in solution at the same concentration but at varying temperatures, to the effect that with rising temperatures their activity is correspondingly increased. This law has been found to apply to bodies of the nature of enzymes, toxins, etc. Thus, for example, the activity of invertase in the conversion of cane-sugar into invert-sugar increases with increasing temperature until a point is reached at which physical conditions, such as precipitation of the enzyme, lead to the suspension of its function and to a decrease in activity.

The recent work of Arrhenius and Madsen[14] on the hæmolysis of red blood corpuscles, making use of dilute solutions of toxins, such as diphtheria and tetanus, has demonstrated that these bodies exert a hydrolytic effect very similar to that of weak acids and bases and following the physico-chemical laws governing dilute solutions both as regards concentration and temperature variations.

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[12] Biernacki, *Pflüger's Archiv*, 1891, xlix, 112.

[13] See Duclaux, Traité de Microbiologie, vol. ii.

[14] Festskrift af Indvielsen ved Statens Seruminstitut, Copenhagen, 1902.

If, as is highly probable, the proliferation of tumor cells is attributable to the presence of a toxic or chemical body acting as a stimulus, the concentration of the body in question most favorable to active proliferation probably lies within comparatively narrow limits, and consequently the slight changes in the concentration of this body would produce a marked variation in stimulus.

The second law considered above regarding the influence of temperature makes it appear probable that increased temperatures at a given concentration would produce a result similar to increase in concentration at the same temperature; in other words, an increase in stimulus to proliferation. Thus, where the concentration is too low to produce a marked effect at normal temperatures, an increased activity may be brought about by increased temperatures, the chemical energy of the stimulating body being thereby increased. On the other hand, the incubation of materials in which the stimulant was already present in the most favorable proportions for the normal temperatures would so increase the activity of the body in question as to exceed the optimum and eventually exert a destructive, inhibiting action upon the development of the cells themselves.

That so short a treatment in saline solutions at temperatures equal to or not far in excess of the normal should produce so marked an effect may at first sight appear impossible, but the effect of the highly dissociated saline solution employed must not be ignored, and for rapidity of effect we have a perfect analogy in the results published by Calkins[15] on the proliferative energy of paramœcium. In certain of his series which had been maintained for considerable periods of time on a uniform diet and were consequently relatively inactive, the effect of subjecting them for a period of twenty-five to thirty minutes to the influence of such salts as potassium phosphate and potassium chloride, and once more returning them to the original medium was to increase greatly their rate of subdivision for a considerable period exceeding in time one or even two generations of our mouse tumors.

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[15] *Journ. Exper. Zoöl.* 1904, i, 423.

A careful consideration of the theory elaborated above may be made to throw much needed light on the perplexing problems which daily present themselves in studying cancer, more especially in connection with the transplantation of mouse tumors. Enzymes and toxins, like catalytic chemical agents, exhibit, as stated above, marked variations in activity with varying temperatures. Toxins and poisonous chemical bodies are capable at extreme dilution and within very narrow limits of concentration of exerting a marked stimulation upon the cell. We have ample evidence of the existence of a stimulating agent in the cancer cell. If this body is possessed of a chemical or enzymotic nature, it is only to be expected that incubation of the cells containing it, in dilute saline solutions, would lead to its liberation and transformation into a temporarily more active state. Such a stimulus, even applied for an extremely short time, is capable, as shown above, of exerting a prolonged effect on certain organisms. In our experiments the stimulating agent may be said to be present at optimum concentration for producing proliferation in those tumors which on direct inoculation produce yields of 80 per cent. and upwards. Consequently any increase in temperature may be expected to exert destructive action, the stimulating body thereby reaching a degree of activity which would lead rather to the destruction than to the stimulation of the cells. On the other hand, tumors possessed of a less marked proliferative energy, which have grown less rapidly, shown less increase, and yield a smaller percentage on direct inoculation, may be stimulated to produce an optimum effect at a temperature slightly above the normal; whilst those which under normal conditions give extremely small or practically no yield may be stimulated by even higher temperatures, the activity of the relatively small concentrations of the agent in question being so increased by incubation as to lead to a relatively increased yield after such a treatment.

That chemical stimuli exert an effect only within very narrow limits of concentration and only in the presence of suitable solvents may well account for the extraordinary difficulties sometimes experienced in obtaining successful and uniform transplantation of tumors. It may account for variation in the speed of development of individual tumors in the same series, for spontaneous recoveries, etc.

In conclusion, we may once more draw attention to the fact that those tumors which apparently contain excessive quantities of the body in question, are those which have recently reached maturity, have partially undergone necrosis, and contain relatively large proportions of calcium. The compounds of complex organic bodies with calcium are, for the most part, relatively insoluble as compared with those of potassium and sodium. It is conceivable that this extraordinarily rapid necrosis, without intervention of bacteria, of those tumors derived from the most virulent series associated with a preponderance of calcium over potassium,[16] is a reaction on the part of the organism whereby the active stimulating agent is at least for the time being removed from the scene of action by precipitation.

In such a case it is conceivable that the influence of incubation in a dilute saline solution is to convert the stimulating agent into an active, soluble form capable of exerting an effect upon any susceptible cells subjected to its action.

Such a stimulating agent as that referred to above is obviously not generated spontaneously, and it would be interesting to trace its possible source by observing to what extent the above results can be made to harmonize with the theories generally advanced regarding the origin of cancer. Owing however to the paucity of our present material it would be preferable to postpone the consideration of this question until we have more experimental data at our disposal.

[16] Clowes and Frisbie, *loc. cit.*

# STUDIES UPON CALCAREOUS DEGENERATION.*

## V.—THE RELATION OF EXPERIMENTAL ARTERIAL DISEASE IN ANIMALS TO ARTERIOSCLEROSIS IN MAN.

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PLATE XXIV.

Since Josué first produced arterial changes in rabbits by means of adrenalin there has been a whole host of experimenters who have repeated and confirmed his work. Although Josué was the first to produce arterial lesions by means of adrenalin, he was preceded by Gilbert and Lion who were successful in bringing about arterial changes, by means of bacteria and their toxins; these lesions they believed simulated arteriosclerosis in man. Sumikawa, too, caused inflammatory changes near the arteries in rabbits, and believed that the vascular changes were identical with those found in arteriosclerosis. However, there is no doubt that arterial changes have been produced with most success by the inoculation of adrenalin. Erb, Baylad and Albarède, Külbs, Fischer, Scheidemandel, and Lissauer have experimented with adrenalin, and have noted the vascular changes. In the main their observations agree, but details of minor importance have been emphasized by some of them. B. Fischer has further found that digitalin when inoculated intravenously produces lesions similar to those caused by adrenalin.

The French workers, Josué and Baylad and Albarède, are most emphatic in maintaining that the intimal changes found in the vessels of experimental animals are of prime importance. The others, with whom I agree, hold that the medial lesions are the principal changes caused by adrenalin. The intimal changes,

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* This study was carried out at McGill University, Montreal, under a grant from the Rockefeller Institute for Medical Research.

which are rarely found, are of a secondary nature, or else are brought about by a process quite distinct from that producing the medial changes.

On account of the importance of a proper interpretation of these experimental lesions, and of the relation which they bear to the diseases of the arteries in man, I feel justified in making a histological comparison of the experimental and human lesions. It is impossible to call the macroscopical changes in the rabbit's aorta atheroma or arteriosclerosis unless the histological examination supports this view. I do not doubt that some have been misled in the use of these terms by the naked-eye appearance of the intima, while others have used the term arteriosclerosis in its broadest sense. Should the latter course be adopted, it should be recognized that experimental arteriosclerosis in rabbits is entirely distinct from sclerosis of the aorta in man. Moreover, as I shall point out later, the term atheroma should not be used in connection with the adrenalin lesions.

It is unnecessary for me to give in detail the methods of experimenting on animals, further than to state that my methods resemble those of previous investigators and more particularly those described by B. Fischer. The pure solution of adrenalin chloride, one in a thousand, was injected into the ear veins of rabbits.

Microscopical lesions, such as dilatation of the blood-vessels, hæmorrhages in the brain, and aneurismal distentions of the larger vessels, were noted. The most frequent lesions were situated in the descending arch of the aorta, where white calcified plaques formed the chief naked-eye alterations. The aneurisms were found to be situated in the centres of these white plaques which have been described by some as atheromatous areas. Histological examination shows that these plaques, at an early stage, are situated entirely in the media and that the calcification which occurs here is the secondary result of a degenerative, chiefly fatty, change which takes place in the media. Calcification is never found in the intima, and only rarely are there any intimal changes. The intimal changes which occur are of two kinds: either there is a local heaping up of endothelial cells at a point where no degenerative changes of any kind have taken place, or there is thickening of the intima underneath the endothelial cells, chiefly due to proliferation of the connective tissue, in which slight fatty changes may occur. Such being the case, the term atheroma must be discarded in describing the experimental arterial lesions in rabbits.

As I have pointed out elsewhere, the primary change in the media, preceding the stage of calcification, is fatty degeneration of the muscle cells, followed by fatty degeneration of the elastic fibres. In the earliest lesions, before any macroscopic change can be noted in the vessels, fine granules of fat are aggregated about the nuclei of the muscle fibres. These granules of fat have an arrangement not unlike that seen in fatty degeneration of the heart muscle. From this stage of degeneration the process advances rapidly so that in a few days the median zone of the middle tunic of the vessels is in an advanced stage of degeneration. Fischer has already noted the rapidity with which the muscle fibres change under the influence of adrenalin inoculation, and hence has applied the name arterionecrosis. Since the degenerative changes are not so rapid as to prevent the appearance of fatty degeneration, and since there proceeds with the apparent necrosis a process of calcification, the name proposed by Fischer is a little misleading. I must grant that sections stained with hæmatoxylin and eosin give a picture of an active necrosis, but with Weigert's stain alone, or combined with Sudan III, the necrotic appearance disappears.

My studies lead me to accept the histological descriptions given by Fischer and others, except, as I have stated, that I find fatty changes in the muscle and elastic fibres preceding the deposit of the calcium salts. The mode in which these calcium salts are laid down has also been discussed in full in another paper, but as regards the sites of the calcium deposit I wish to speak more fully.

In sections stained with hæmatoxylin and eosin, it is seen that the lesion lies in the middle zone of the media and that in the early stages the muscle fibres become granular and lose their nuclei. With this degeneration of the muscle fibres, which must necessarily be accompanied by softening of the tissue, the elastic fibres are packed more closely together, leaving less room between them for the muscle cells. At these points, where there is a loss of the muscle elements, the vessel wall is distinctly weakened, and partially gives way, leading to small aneurismal sacculations. In advanced lesions these aneurismal dilatations are the most marked macroscopic changes that are found.

When the muscle cells in the media have undergone fatty degeneration, the condition advances, until the cells become fairly loaded with the fat granules. Nuclear degenerations then become apparent and soon the cells die, leaving only their outline marked by the deposit of the fat granules which were in them. These fat granules do not coalesce but remain as isolated particles. Passing through the stage of fatty acids and soaps[1] they are converted into calcium compounds of the fatty acids, and hence the calcium deposit occurs as a fine sandlike material distributed in the sites of the former fatty granules. Following closely upon this change, the elastic fibres also become fatty, as Jores has observed, and go through the same process of calcification as the muscle cells. There is now a fine granular deposit of calcium at the site of the muscle fibres and rigid zig-zag calcified elastic fibres lying between them. This picture is not seen in the advanced stages of the calcification of aorta in rabbits, for the calcium salts have coalesced more or less to form larger masses, so that the former histological structure of the media is entirely lost. In no instance of experimentally calcified vessels in animals has primary calcification of the intima been noted. Occasionally I have seen very advanced experimental arterial disease in which the calcification of the media extended immediately below the intima.

If we are to accept Jores's definition of arteriosclerosis, namely, that the disease consists of a hyperplasia with degeneration of the musculo-elastic layer of the intima as seen in the human aorta, these experimental lesions would not fall in this category. I am not, however, inclined to accept this restricted definition of arteriosclerosis, particularly as regards the human disease.

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[1] See the studies upon calcareous degeneration by the writer. *Jour. of Exper. Med.*, 1905, vii, 633.

Lissauer compares the experimental lesions in arteries of rabbits to syphilitic arterial disease in man, pointing out that syphilitic arteritis attacks particularly the media. He falls short of a proper comparison. The main factor in the experimental lesions is the calcification of the muscle and elastic fibres in the media, and this never takes place in syphilitic disease of the vessels. In syphilitic arteritis the main reaction, consisting of an inflammatory change with new formed blood-vessels, an infiltration of small cells, and later the production of connective tissue, takes place in the outer third of the media. The blood vessels affected by adrenalin, on the contrary, show no reparative reaction in the media, the process being entirely a degenerative one. If a reaction does occur, it is secondary to a medial degeneration, and this reaction consists of a heaping up of the endothelial cells in the intima.

I would point out that although the lesion does lie in the media—and especially in the middle zone of the media—there are no changes in the vasa vasorum. All the hypotheses, maintaining that the action of adrenalin on the vasa in the vessel wall is the prime factor in the production of the necrosis, are based upon general inferences regarding the action of this drug in other parts; the histological appearances give no support to this theory.

A second arterial disease which Lissauer wishes to compare with the vascular changes caused by adrenalin is the so-called neurotic angiosclerosis. He points out that Lewaschew and also Fraenkel found that by injuring or cutting the nerve supply of vessels, they became infiltrated with connective tissue, and that the change lay only in the media. Their results are quite contrary to those of Jores, who found that neither injury to a nerve nor the complete severance of it had any effect on the histological structure of the vessel it supplied. In syphilitic arterial disease, as I have mentioned, and in the neurotic lesion, calcification of the media is never seen.

There is, however, an arterial disease in man which simulates the experimental arterial degenerations in every respect. This disease I have spoken of elsewhere as Moenkeberg's type of arteriosclerosis. This disease, as Moenkeberg and others have pointed out, affects the vessels of the extremities—the vessels of the muscular type—that is, arteries in which the muscle tissue predominates in the media. From this lesion the clinician makes the diagnosis of arteriosclerosis when he finds the radials hardened like "pipe stems" or "beaded like a trachea." It is now definitely known that this form of arterial disease is distinguishable from arteriosclerosis as we find it in the human aorta. Moenkeberg's type of arteriosclerosis affects the media alone, causing degenerative changes. The intima is affected neither by degenerative nor by reparative changes, or if such changes are present they are secondary to or parts of another disease and not associated with changes taking place in the media. This disease of the media affects the vessels of the extremities—the femorals, tibials, internal and external iliacs, radials, and brachials being particularly susceptible.

Preceding the deposition of calcium salts in the media, there are degenerative changes which consist chiefly of fatty degeneration, which is visible to the naked eye, and can be distinctly traced in the media when the vessel is cut open longitudinally. This early fatty degeneration in the media differs from that of arteriosclerosis of the aorta in that the fatty plaques do not stand above the surface of the intima, but can be seen through this membrane. One is convinced by the microscopical picture, that in the early stages the muscle cells undergo a fatty degeneration characterized by the deposition of the finest fat globules. The calcium salts, too, are found to be deposited as fine sandlike granules in the degenerated muscle fibres. In the early stages of degeneration, calcium granules are found in double wedge-shaped aggregations lying between the elastic fibres, and this grade of calcification is not to be recognized by the naked eye. However, with the constant accumulations of fresh calcium deposit, the granules lie very close together and then coalesce. At this stage, when the calcium granules are becoming densely aggregated, we find that the vessel wall decreases in thickness; there is a thinning of the media with a bulging outward of the wall at this point. Distinct multiple aneurismal dilatations are of common occurrence in these vessels, so that the outer contour of the vessel is quite irregular. From the inner surface of the vessel it is seen that the calcified areas form depressions which are lined by smooth and unchanged intima. Microscopically, the elastic fibres are seen to be stretched into straight lamellæ and no longer show the wavy character found in healthy vessels. The elastic fibres, too, are calcified and are lying more closely together, although in advanced cases this is difficult to distinguish, as the calcium deposit in the muscle and elastic fibres have run together into a solid mass. In consequence of the constant tension and stretching of the elastic fibres, they have become calcified in the position that they had in these vessels, and thus we find the straight calcified laminæ. Where calcification of elastic fibres has advanced beyond the general area of calcareous degeneration, and, too, where contractile muscle fibres have persisted between them, these elastic fibres have taken on a zig-zag outline due to fractures.

Microscopically one can demonstrate that the running together of the calcium granules takes place in the middle of the media, and that at either end of such a mass are outrunners of calcified elastic fibres and granular degenerated muscle fibres. The internal elastic lamina is stretched as it passes over the calcified and aneurismal portion of the media.

The intima occasionally shows some reaction over the areas of medial degeneration, the endothelial cells being heaped up. This intimal change is, however, entirely secondary and has nothing to do with the primary cause of the medial change, and further is not a necessary accompaniment of the medial degeneration.

Moenkeberg holds that the adventitial changes are in great part confined to thickening of the intima of the vasa vasorum, but points out that this limitation is not a constant feature in the disease. There are some who claim that the degeneration of the media is due to occlusion of the vasa vasorum causing poor nutrition of the media. However, the changes in the vasa vasorum are too inconstant to allow us to draw any definite conclusion, and, moreover, it is very hard to estimate the amount of change in these small arterioles with no definite wall, especially when they are collapsed and empty.

Pure medial calcification is commonly found as an accompaniment of advanced age, occurring most frequently after the age of fifty. But, as Marchand has pointed out, it occurs also in young individuals. The disease affects males more frequently than females, the ratio being about six to one. It is further of interest that medial calcification occurs in the vessels of those extremities which are most active; thus in right-handed individuals it is found more pronounced on the right side, while in those whose duties keep them constantly on their feet the femorals are most affected. Again, the disease is particularly common among the laboring classes.

When the experimental lesion in animals and the medial arteriosclerosis in man are compared, they present great similarity in their anatomical, histological, and, it may be, their etiological characters. In each case the essential lesions are confined to the media, the changes in the intima being of a secondary nature or else the result of a different disease. In both lesions the process in the media rapidly leads to calcification of the muscular and elastic elements, fatty change preceding the deposition of the calcium salts. Aneurismal dilatations at the sites of the calcium deposits in the media often occur with both lesions, and in the dilated part the vessel wall is much thinned as the result of the loss of muscular elements and the packing together of the elastic fibres.

Both diseases are brought about or accompanied by an increased blood pressure; in the one it is artificially produced by the use of adrenalin and involves the general circulation, while in the other it is localized in parts, the extremities, where increased work, straining and constriction of the muscles, lead to heightened pressure. Whether this heightened blood pressure acts directly on the vessel walls to cause the medial degeneration, or whether by the stretching of the arteries the vasa vasorum are in part or wholly pinched off, leading to focal necrosis in the media, is not wholly clear. Certainly from the focal character of the experimental lesions and from the annular arrangement of the degeneration in the vessels of the extremities in man, I should feel very much inclined to think that the vasa played an important rôle in the production of these changes. However, we must not lose sight of the fact that in the experimental animals the toxic character of the substances inoculated may be an important factor in the production of the lesions, as Fischer has pointed out.

Both of these lesions must be distinguished from the intimal form of arteriosclerosis as found in the aorta, and the presence of one condition does not indicate the presence of the other.

Several clinicians have made the observation that when pure medial arteriosclerosis is present in the vessels of the extremities without any changes in the aorta there is no hypertrophy of the left heart, while, on the contrary, if the aorta is affected with arteriosclerotic changes the heart shows hypertrophy. This fact suggests that while the aorta is healthy and elastic, it relieves, to a great extent, the increased blood pressure which would naturally follow the hardening of the peripheral vessels, while, on the other hand, if the elasticity of the aorta is destroyed, the increased pressure is directly transferred to the heart, leading to hypertrophy of the organ. The experimental hardening of the walls of the aorta apparently confirms this view, since hypertrophy of the heart frequently occurs with it.

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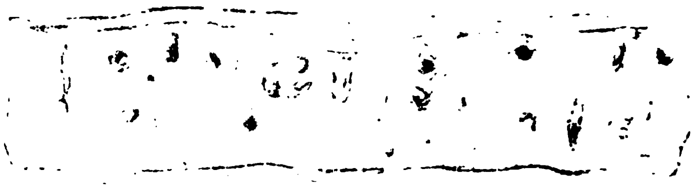


Fig. 1.

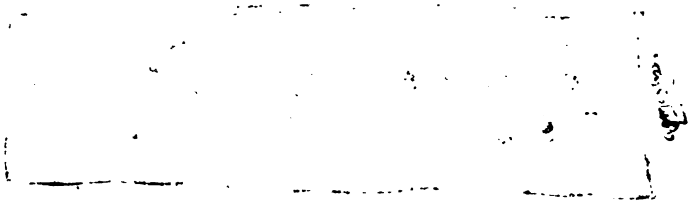
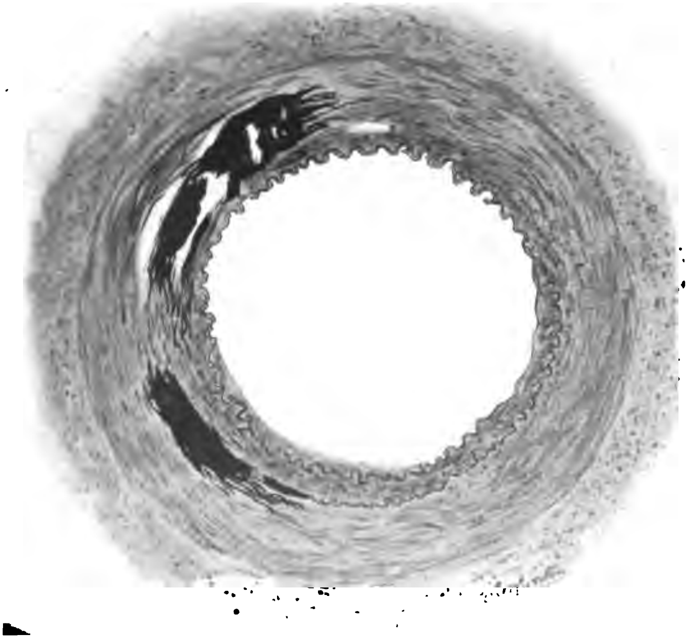


Fig. 2.



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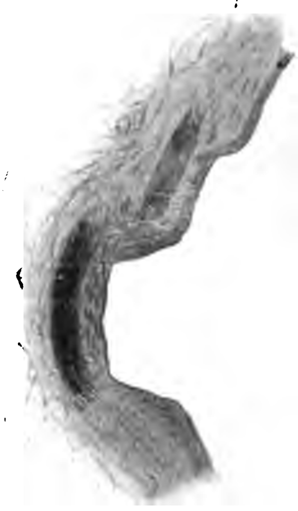

Fig. 4.

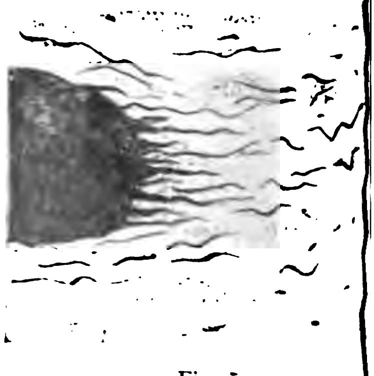

Fig 5.

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Sumikawa.—*Ziegler's Beiträge*, 1903, xxxiii, 242.

## EXPLANATION OF PLATE XXIV.

Fig. 1.—Femoral artery (natural size), showing pittings and small aneurismal dilatations in the areas of calcification of the media.

Fig. 2.—Aorta of rabbit (enlarged x 2), after injections of adrenalin, showing small calcified plaques with depressions and aneurismal bulgings in their centres.

Fig. 3.—Femoral artery (low power, Leitz obj. 3, ocular No. 2), showing calcification of the media with little change in the intima other than splitting of the internal elastic lamina.

Fig. 4.—Aorta of rabbit after injections of adrenalin (low power), showing calcification in the media with no change in the intima. Vessel wall shows aneurismal dilatation.

Fig. 5.—Femoral artery (high power), showing densely calcified media, along with a less intensely calcified area in which the elastic and muscle fibres are in the early stages of calcification.

# THE PATHOGENESIS OF EXPERIMENTAL COLITIS, AND THE RELATION OF COLITIS IN ANIMALS AND MAN.

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There is no longer any real doubt that in the group of dysentery bacilli we possess the essential causes of the dysenteric inflammations of the intestine in man. This general statement is justified notwithstanding the facts that in man a class of inflammations of the intestine is caused by pathogenic amœbæ, and an indefinite group of intestinal inflammations, due to no common cause, is occasionally met with. The morbid conditions produced, on the one hand, by pathogenic amœbæ, and, on the other, by irritating foreign bodies, the bacteria of food poisoning and the salts of heavy metals, are, taken together, not very infrequent; but as compared with those caused by the dysentery bacilli they are relatively inconsiderable.

The experimental studies of the past ten or twenty years have shown that a considerable number of diverse poisons produce, in animals, inflammations of the intestine. Some of these poisons are of known definite composition, as the salts of mercury and some other metals; others are of indefinite nature, so far as our present knowledge reaches, as, for example, the peculiar poison contained in putrefying fluids. A third class of poisons of somewhat more definite nature, capable of causing inflammations of the gut, are of vegetable origin: these are the toxic products of bacterial growths, ricin, abrin, etc. While the precise chemical nature of these toxic bodies is still unestablished, the poisons themselves are sufficiently defined to permit of their employment under uniform conditions of experiment. The present paper deals with the effects produced on rabbits by inoculation, chiefly, of a toxic product obtained from the Shiga dysentery bacillus, and a study of the manner in which the peculiar effects are brought about. In the course of the investigation it was found necessary to resort to the use of some other poisons, *e.g.*, corrosive sublimate and ricin, in order to bring out certain facts which the use of dysentery toxin had failed to establish, or to establish, beyond doubt, certain deductions drawn from the experiments with the dysentery toxin. The results of this experimental study are presented here because of their bearing upon the pathogenesis of colitis in man.

Two main groups of dysentery bacilli are now recognized: the "Shiga" and the "Flexner" groups. The first agree with the bacillus first isolated by Shiga from cases of Japanese dysentery; the second isolated by one of us (Flexner) from cases of Philippine dysentery. The two groups possess in common the capacity to set up dysentery in man. No profound distinction in this power can be drawn between the bacilli. The disease which they cause appears in sporadic, endemic, and epidemic forms. In the case of young children it would appear, from our present knowledge, that the Flexner type of dysentery bacillus causes much more frequently than the Shiga type certain varieties of ileo-colitis. The cultural distinctions between the two groups are considerable: the Shiga type of bacilli has a restricted power to ferment the sugars, while the Flexner type has a rather wide capacity of fermentation. The two groups are also separated by their agglutination reactions: they are less widely separated in respect to their reactions to immune sera. On the other hand, they are sharply separated by their capacity to yield, in fluid cultures, soluble toxins. The Shiga type of bacillus readily produces a soluble toxin, the Flexner variety either not at all or in minimal quantities. Shiga bacilli grown on solid culture media, killed at 60° C. or with chloroform, still yield, when suspended in water or salt solution, a toxin which goes over into the fluid. The Shiga bacilli easily undergo autolysis through which the toxin is liberated. The Flexner bacilli do not yield by autolysis, any more than in fluid culture, a toxic body to a surrounding fluid medium.

As tested on certain small laboratory animals—young guinea-pigs, for example—the two types of bacilli are about equally toxic. This toxicity does not depend, necessarily, upon multiplication of the bacilli, for the dead micro-organisms are toxic. Hence the Flexner type of bacillus must yield in the animal body an active toxic product. As tested on rabbits, however, the Shiga type of bacillus is more toxic than the Flexner type. The rabbit is, indeed, very sensitive to the action of the Shiga dysentery bacillus or its toxin. The easily demonstrable effects are exerted upon the central nervous system and upon the intestine. Two kinds of intestinal lesions occur either separately or combined. The more common lesions appear in the small intestine: the gut is dilated, the vessels are injected, the Peyer's patches swollen and congested, and a faintly greenish-yellow glutinous or serous fluid fills the lumen. Although occurring less frequently the lesions in the large intestine are much more striking. The part of the large gut affected is, almost exclusively, the dilated cæcum and appendix. Rarely is the colon involved. The lesions vary in intensity: the coats of the gut are greatly thickened by inflammatory œdema, in which case the mucosa is yellowish-white and thrown into deep folds and corrugations, or with the œdema more or less hæmorrhage may be associated. At another time the transverse folds of mucous membrane are affected chiefly; they are swollen, the edges are hæmorrhagic, and pseudo-membrane is scattered over the surface. Or, again, the transverse folds are greatly and the intervening mucosa is less affected, and patches of swollen and hæmorrhagic mucous membrane, covered with a false membrane, appear upon and between the folds. The hæmorrhage may extend into the serous coat. It is impossible, in a brief space, to describe all the variations of appearance of the affected mucous membrane. There is, however, in many cases striking similarity in the lesions, as regards location and general pathological appearances, with the early diphtheritic dysenteric lesions in man. The masses of lymphoid tissue at the ileo-cæcal valve and in the appendix are invariably swollen in the rabbits, showing the large intestinal lesions, and frequently they are beset with small hæmorrhages. The mesenteric lymphatic glands are always swollen, œdematous, and congested.

There are considerable variations of susceptibility to the dysentery toxin among the rabbits. A part only of the rabbits which succumb to the toxin develop the nervous and large intestinal lesions. A given lot of toxin will cause in certain rabbits the nervous and intestinal lesions, and in still other rabbits transitory illness from which they recover, or death without visible lesions. Neither does there appear to be a marked relation between dosage and lesions, or even death. Of a given toxin 0.01 cubic centimeter may be fatal to one series of rabbits while another series may survive 0.1 cubic centimeter of the same poison, the weights of the rabbits having been about equal. Larger doses usually kill, but the period of survival varies considerably. The symptoms which follow the inoculations are diarrhœa, and paralysis and convulsions. The diarrhœa is sometimes bloody. The paralysis begins in the upper extremities and extends, at times, to the lower extremities. It may be incomplete. The animals may survive for days after the extremities are paralyzed. They lie on the side with the head drawn back, and in opisthotonic position. The convulsions are irregularly intermittent, and may be excited by disturbance of the animal. Towards the end of life the paralysis of the extremities becomes more marked. In general appearance these animals resemble rabbits in the paralytic stage of rabies. Although rabbits may survive many days after the onset of the paralytic symptoms, which may themselves be several days in developing, yet in some instances the symptoms of paralysis may appear within twenty-four hours and the animals survive this appearance only a few hours. Speaking generally the larger doses of toxin —0.5 to 1 cubic centimeter—produce marked symptoms and cause death earlier than the smaller doses.

The dysentery toxin which we are considering was first obtained by Conradi, whose results were briefly mentioned by v. Drigalski[1] and later were fully described by himself.[2] The method employed by Conradi consisted of aseptic autolysis of the cultures. Of his preparations 0.1 cubic centimeter was fatal, cent. salt solution, the organisms killed by exposure to 60° C. for one hour, and the suspension kept at 37° C. for varying periods. The toxic solution was obtained by filtration through a Berkefeld filter. The filtrate was preserved in the ice-box under toluol. The injections were made intravenously in rabbits.

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[1] V. Drigalski.—*Veröffentlichungen der Medizinalabtheilung des Königlich Preussischen Kriegsministeriums, Berlin, 1902, Abt. 2, 107.*

[2] Conradi.—Ueber lösliche durch aseptische Autolyse erhaltene Giftstoffe von Ruhr- und Typhusbazillen. *Deutsche med. Wochenschr.*, 1903, xxix, 26.

Although aside from our main theme, which was to ascertain the manner in which the intestinal lesions in rabbits were produced, a series of experiments on the comparative toxicity of living, dead, and autolysed cultures was made. Our results do not uphold Kraus and Dörr who state that an active toxin can be obtained without autolysis, and they indicate, further, that the separated toxin is more effective than the same quantity of poison contained within the living or the dead bacilli.

TABLE I.

| Series No. | Weight in grams | Amount injected | Nature of material injected | Results |
|---|---|---|---|---|
| 26 | 2000 | 0.1 c.c | living suspension | dead after 10 days |
| 27 | 1620 | " | " " | lives |
| 28 | 1920 | " | suspension after 1 hr. at 60° C. | lives |
| 29 | 1270 | " | suspension after 1 hr. at 60° C. | dead after 5 days |
| 30 | 1150 | " | suspension autolysed 3 days | dead after 11 days |
| 31 | 1150 | " | suspension autolysed 3 days | dead after 2 days |

TABLE II.

| Series No. | Weight in grams | Amount injected | Autolysed — days | Results |
|---|---|---|---|---|
| 23 | 1610 | 0.5 c.c. | 2 | lives |
| 24 | 1570 | " | 3 | dead after 2 days |
| 25 | 1740 | " | 4 | lives |
| 32 | 2470 | " | 5 | dead after 5 days |
| 33 | 1270 | " | 6 | " " 5 " |
| 34 | 1300 | " | 7 | " " 2 " |
| 35 | 1300 | " | 8 | " " 2 " |
| 38 | 1700 | " | 9 | lives |
| 39 | 1310 | " | 10 | dead after 3 days |
| 66 | 1160 | 1.0 c.c. | 15 | " " 2 " |
| 75 | 900 | " | 27 | " " 6 " |

Since Conradi believed that the toxin may be weakened by allowing the autolysis to continue longer than forty-eight hours, an experiment was made to determine this point. As Table II shows the toxin is still effective after a period of autolysis extending to twenty-seven days.

The preceding series of experiments serve also to confirm the statement already made, namely, that individual peculiarities play an important part in determining susceptibility to the poison. Of several rabbits the larger may succumb and the smaller escape, or those receiving the smaller doses may die and those a larger dose survive. But where very large doses, from two to five cubic centimeters, are injected, the animals much more rarely survive; and under these circumstances the animal receiving the largest dose usually succumbs the earliest.

The tendency of the diphtheritic lesions of the intestine to confine themselves to the cæcum and colon led early to the notion that retention of the fæces was the chief agent in causing the condition. All the earlier views are summed up in Virchow's paper which appeared in 1863 in which he defined his own belief that fæcal retention is a necessary condition to the production of diphtheritic dysentery. This view now dominates in the pathology of dysentery. It will be instructive to present, in abstract, Virchow's[11] argument in order that a comparison may be made of the conditions present in dysentery in man with those occurring in the experimental disease in the rabbits.

> "Dysentery arises from the action of products of decomposition upon the intestinal mucosa already the seat of a superficial catarrhal inflammation. That this is true is shown most clearly by the manner of extension of the dysenteric process. The projecting points—those which come directly and remain longest in relation with the contents of the intestinal mucosa—are first and most severely affected. Rokitansky is mistaken in saying that as a rule the dysenteric process extends from the valve to the rectum, taking on in intensity from above downwards. Generally speaking those portions of the large gut of which the contents remain longest in contact with the mucosa are most severely affected. Those parts are the cæcum, the hepatic, splenic, and sigmoid flexures, and the rectum. Should the intestine present abnormal flexures then the rule holds for them, should the normal flexures be accidentally wanting then this part of the

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[11] *Virchow's Archiv*, 1863, v, 348.

gut remains relatively free of disease. In particular it is the projections of the mucous membrane, caused by the anatomical distribution of the muscle tissue, which are affected by preference. Hence the especial susceptibility of the mucosa along the insertion of the three longitudinal muscle bands, and of the transverse folds of the membrane. In that the diphtheritic process establishes itself first on the projecting and later on the intermediate mucosa, there arise those regular geographical figures which indicate the intensity and mode of spread of the lesion. When it is considered that the diphtheritic process does not begin regularly at a definite portion of the mucous membrane, as, for example, the rectum, but that this portion is often least involved while a higher level of intestine is often intensely affected, then the presumption is difficult to set aside that the pathological process is due to some agent which, acting locally, causes the lesions.

"Rokitansky (*Pathological Anatomy*, ii, p. 265) compares with right the anatomical changes in dysentery with those which caustic acids produce; and hence the question arises whether the necrosis is due to a direct chemical change in the tissues caused by an action starting from the surface. Dysentery begins undoubtedly, as is the case with every superficial inflammation of the mucous membrane, as a simple catarrh, the lesions being met with only after the diphtheritic condition has appeared. Hence it is necessary to ascertain whether the increase from catarrh to diphtheria takes place without the action of local injurious agencies. It may in general be assumed that a simple intestinal catarrh, by reason of the presence of products of decomposition, is converted at those points where decomposition is going on into a diphtheria; and these products are contained chiefly in retained fæcal masses."

In his paper on dysentery published in 1877 Virchow[12] states that diphtheria of the intestine may exist independently of dysentery and instances his findings in some cases of Asiatic cholera of diphtheritis of the intestines. What is of special interest, in this connection, is the statement that the diphtheritic process may involve the ileum, affecting partly the surface of Peyer's patches and partly the villi. "In the latter cases the mucosa was much thickened, firm, and disposed in high transverse folds, making it appear at first as if it were jejunal mucosa.

The increase in our knowledge since these writings has yielded the tangible agents of dysentery and set aside the notion of the action of the products of decomposition, unless, indeed, the toxic constituents of the dysentery bacilli be viewed as such products. Although we have secured this advantage of knowledge, no direct application of it to the pathogenesis of the lesions of dysentery has been attempted. It is clear from the descriptions of the experimenters with dysentery toxin that the intestinal lesions in rabbits agree very closely with those of human dysentery. The small intestine is found constantly, or nearly so, in a state of catarrhal inflammation, and in a percentage of animals the large gut shows diphtheritis, œdema, and hæmorrhage.

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[12] *Charite Annalen*, 1877, ii, 706.

None of the previous experimenters seems to have been impressed with the finer localization of the lesions in the large gut of the rabbit. Aside from the statement that the cæcum is the seat of predilection nothing is recorded. An analysis shows, however, that two main sets of lesions are encountered. In one the parts of the intestine chiefly affected are the transverse folds of the mucous membrane of which the tips or the entire fold may be involved. In some instances the intervening mucosa escapes, in others the pathological process appears there also, often as focalized areas, but sometimes as a more or less diffuse involvement. In the other a diffuse involvement of the cæcum appears to have been present from the start. The former cases present many points of similarity with the appearances described in human dysentery; while for the latter cases it may be said that in the Philippine Islands one of us (Flexner)[13] observed in autopsies upon American soldiers who died of dysentery, diffuse, hæmorrhagic lesions which agree essentially with the diffuse hæmorrhagic condition present in the rabbits.

The importance of this correspondence appears when it is considered that in man the noxious agent reaches the intestine directly through ingested materials, while in rabbits the poison is injected into the circulation and must reach the intestine through its blood supply. The conclusion, in the case of the rabbits, seems sufficiently obvious; the toxic material is secreted into the intestine and produces directly or indirectly pathological effects. But whether in man the noxious material acts directly or through the blood remains as before an open question.

The rabbit is not subject to infection with the dysentery bacillus when cultures are fed the animal; and we have found that injection of cultures of the bacilli directly into the duodenum is apparently without effect in this animal. As was to be expected from this result the injection into the duodenum of considerable quantities (up to 10 c.c.) of the autolysed toxin is also without action. The rabbit is not, therefore, subject to poisoning with the dysentery bacillus or its toxic products when they are brought at once into the gastro-intestinal canal. All other forms of inoculation—subcutaneous, intraperitoneal, intravenous—are effective. It may therefore be assumed that direct contact of the bacilli or the toxin with the mucosa of the rabbit's gut cannot lead either to absorption unchanged or act injuriously upon the membrane. Since it was possible that the poison might be altered in the small intestine and reach the cæcum in an inactivated form, an experiment was carried out to ascertain whether direct contact of the toxin with the mucosa of the cæcum would lead to a different result. A rabbit was starved and given magnesium sulphate per os to empty the bowel. The peritoneum was opened and 1.0 cubic centimeter of dysentery toxin diluted with ten cubic centimeters of salt solution was injected directly into the cæcum and allowed to come into contact with the mucous membrane. No immediate effect was noted, and the animal showed, on being killed forty-eight hours later, no lesions of the intestine. The interpretation to be placed upon this experiment is clear, and all the experiments taken together would seem to indicate that the lesions of the gut present in the rabbits given the dysentery bacillus or its toxin, in such way as to bring the poisons into the blood, are caused not by direct effect upon the surface of the mucous membrane but indirectly through the act of elimination of the poison by the intestinal mucosa. In endeavoring to explain the absence of action of the toxin upon the intestine when given by mouth or injected into the lumen of the gut, tests were made on the resistance of the toxin to peptic and tryptic digestion, but no light was obtained. It was found that the toxin is destroyed by peptic digestion (artificial gastric juice) after twenty-four hours' contact, while 0.3 per cent. hydrochloric acid alone is without influence on the toxicity. On the other hand, the toxin resists tryptic digestion for eighteen hours, but after a longer contact (at the end of three days) it is destroyed. The addition of solutions of erepsin, prepared by Cohnheim's method, to the trypsin does not accelerate the effects.

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[13] On the Etiology of Tropical Dysentery, *Philadelphia Medical Journal*, 1900, vi, 414.

The toxin is moderately heat stable. Neisser and Shiga found that a temperature of 75° C. reduced greatly its action; Vaillard and Dopter state that the temperature of 70° to 75° C. maintained for one hour is without effect, while at 75° to 80° C. the toxicity is diminished and at 81° C. destroyed. Rosenthal's specimen of toxin was weakened by heating to 70° C. Our results agree with those of Vaillard and Dopter: the toxin studied by us was injured at 80° C. and destroyed at 81° C., those temperatures having been maintained for one hour.

It was noted in some earlier experiments by one of us (Flexner) that when rabbits are injected intravenously with the dysentery bacillus the living bacilli may be found in the bile. Notwithstanding the negative results obtained from direct injection of the bacilli and the toxin into the intestine, it was considered desirable, in view of the possibility of the intestinal lesions being eliminative in nature, to exclude not only the bacilli but also the entrance, possibly, of the toxin into the intestine with the bile. To accomplish this exclusion two methods were employed: the common bile duct was tied; and a biliary fistula was established. After ligating the duct in two places the portion lying between the ligatures was resected. A permanent biliary fistula was made by inserting a special cannula into the apex of the gall bladder and conducting the secretion through rubber tubing to the exterior. The common duct was always tied off. The operations were carefully conducted with all precautions and infection was readily avoided. Table III shows the somewhat surprising results of this series of experiments. While of the ten control animals five developed the characteristic lesions of the cæcum, of the eight animals in which the duct was ligated two only showed similar lesions, and of the ten animals in which a permanent fistula was established only one animal (Number 19) developed lesions of slight degree. As regards rabbit Number 35 it is safe to assume that the small hæmorrhage noted was caused by manipulation of the gut at the operation.

TABLE III.

| Series No. | Weight in grams | Amount injected | Death after—days | Result as regards the lesions of the cæcum |
|---|---|---|---|---|
| Controls | | | | |
| 81 | 1555 | 5mg.powdr.* | 2 | no lesions |
| 83 | 1590 | " | 11 | œdema and congestion, no hæmorrhages |
| 5 | 1300 | 0.5c.c.toxin | 5 | marked œdema, no hæmorrhages |
| 6 | 1630 | 0.5; 0.5; 2.0 | lives | |
| 8 | 2100 | 5.0 | 2 | no lesions |
| 9 | 2200 | 5.0 | 2 | numerous hæmorrhages, with necroses |
| 12 | 2150 | 2.0 | 1 | no lesions |
| 14 | 1570 | 2.0 | 2 | no lesions |
| 16 | 1540 | 0.5 | 4 | very extensive hæmorrhage and necrosis |
| 20 | 2100 | 5.0 | 2 | scattered hæmorrhages and necrosis |
| Tied duct | | | | |
| 82 | 1510 | 5mg. powdr. | 9 | no lesions |
| 84 | 1605 | " | 9 | œdema and hæmorrhages |
| 1 | 1240 | 0.5;1.0;0.5 | 7 | no lesions |
| 2 | 1500 | 0.5;1.0 | 3 | no lesions |
| 10 | 2140 | 5.0 | 3 | no lesions |
| 11 | 2140 | 2.0 | 1 | no lesions |
| 15 | 1520 | 2.0 | 4 | œdema, no congestion, no hæmorrhages |
| 17 | 1740 | 0.5 | 4 | no lesions |
| Permanent biliary fistula | | | | |
| 3 | 1230 | 0.5 | 2 | hyperæmia, no focal lesions |
| 4 | 1530 | 0.5;1.0 | 4 | no lesions |
| 13 | 1880 | 2.0 | 1 | no lesions |
| 18 | 1860 | 2.0 | 3 | no lesions |
| 19 | 2020 | 0.5 | 4 | slight œdema, no hæmorrhage. |
| 21 | 2140 | 5.0 | 2 | no lesions |
| 27 | 2070 | 10.0 | 3 hrs. | no lesions |
| 31 | 2500 | 5.0 | 1 | no lesions |
| 35 | 2100 | 1.0 | 1 | area of hæmorrhage at pt. of cæcum nearest liver |
| 51 | 2300 | 5.0 | 3 | no lesions |

In discussing these results it is perhaps desirable to point first to their apparent paradoxical nature. While the direct injection of dysentery toxin into the intestine is followed neither by death of the animal nor development of visible intestinal lesions, the exclusion of the toxin from the lumen of the gut by cutting off the entrance of bile tends to prevent the lesions without, at the same time, preventing the fatal action of the poison. The nervous symptoms develop in animals in which the bile duct has been tied or a biliary fistula established. It would be wrong to conclude that the toxin exercised no influence on the body merely because the intestinal lesions were absent, for in a part of the fatal cases the striking lesions of the cæcum do not appear; and the less striking ones of the small bowel might well exist and be followed by recovery. The test of action is, indeed, a lethal result or the development of the nervous symptoms; for although some of the animals injected with small amounts of the toxin recover, yet none of those receiving into the circulation as large doses as may be introduced in the intestine without producing severe symptoms ever survive.

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* Made by drying in thermostat at 37° C. the autolysed culture fluid.

Hence one is brought to the conclusion that the poison which acts injuriously upon the intestine, through elimination, is also excreted by the bile. Indeed, it would agree best with the experimental results to conclude that of all the circulating poison at any one period a fraction only is eliminated by the intestinal wall, while the larger fraction passes out of the circulation through the liver. This larger fraction, entering the intestine with the bile, is then partially or wholly reabsorbed, only to be subjected in turn to the same series of events. According to this view the intestinal lesions are gradually developed by successive acts of excretion. It does not follow that this elimination takes place only in the cæcum, and that the absorption occurs only higher up in the gut. While it seems probable that the chief absorption does occur in the small intestine, the existence of lesions in this part of the intestine may point to it as being also, although in a minor degree, concerned with excretion, while there is nothing to prove that absorption may not also take place in the large intestine. No explanation is at hand for the failure of the toxin to be absorbed when introduced in mass into the small intestine. After this relation of the poisoning to the bile secretion into the intestine became apparent, we attempted, without success, to reproduce some of the symptoms of the intoxication by mixing the toxin outside the body with rabbit bile and injecting the mixture into the small intestine.

When the bile is drained away a large part of the toxin is lost, but there still remains available, from the multiples of lethal doses injected, sufficient to cause death through the nervous injury. That the chief intestinal lesions should in these experiments be absent is not remarkable. It is surprising, however, to find that the lesions of the large gut occur more infrequently in the animals in which the bile duct is tied off than in animals not operated upon. It is true that the lesions of the small intestine have been present in some of these animals; and from this it has been concluded, first, that some excretion of the toxin occurs in the small intestine, and, second, that the liver holds back, from the blood, the accumulated toxin which it naturally would secrete into the bile. Whether the poison is merely stored in the liver or destroyed there has not been determined.

The experimental results stated, have brought us to the conclusion that dysentery toxin is both excreted by and absorbed into the blood from the intestine. In this respect the toxin is brought into conformity with certain organic (morphia) and inorganic (iron, manganese) substances which are both absorbed and excreted by the gastro-intestinal mucosa. But interesting as this fact taken by itself may be, our special object at present is to show, as far as possible, the extent to which this property of the toxin of being absorbed and excreted by the intestine may account better than the older views, emphasized by Virchow, for the development of the catarrhal and diphtheritic lesions of dysentery in human beings. The bacteriological studies of cases of dysentery in man examined post-mortem have shown the dysentery bacillus to be present throughout the intestinal tract. It is not limited to the large intestine, although it is, possibly, more abundant in the fibrinous exudations. All parts of the mucous membrane of the intestine can be made to yield the bacillus in culture irrespective of whether they are the seat of pathological changes or not. Since the development in any numbers of the dysentery bacillus does not take place outside the intestinal tract, it must be concluded that the toxin, which causes the lesions and symptoms of the disease, is produced in the gut. It would agree well with the experimental results related to assume that in man the absorption of the dysentery toxin takes place in the small, and possibly also in the large intestine, but that, usually, the chief elimination is by way of the large intestine; and only under unusual conditions, as in the rare cases of diphtheritis of the small intestine, to any great extent by way of the small gut. This general statement of the manner of production of dysenteric lesions, the diphtheritic ones especially, in man does not exclude the possibility that certain secondary causes contribute to determine the extent of the lesions of the large intestine, among which retained fæcal masses might play an important part.

On account of the variable action of the dysentery toxin upon the cæcum of rabbits, the need was felt of studying, by the methods employed in these experiments, more definite chemical substances which provoke eliminative lesions of the intestine. For this purpose corrosive sublimate and ricin were chosen. The lesions of the large intestine which follow the subcutaneous injection of corrosive sublimate in rabbits have been accurately described by Heilborn[14], and those which result from ricin injections into the circulation by Ehrlich[15] and Flexner[16]. Mercury colitis is such a well-known condition that no special description of it is called for, but the fact may be mentioned that the small intestine escapes injury, while the cæcum and the first part of the colon are the seats of hæmorrhage, necrosis, diphtheritis, and ulceration. The most constant and most pronounced lesions are in the spiral folds of the cæcum. The striking lesions in ricin intoxication are general hyperæmia of the the small intestine associated with an excessive secretion of serum and mucous, and swelling of and punctiform hæmorrhages into the Peyer's patches. The lymphatic glands generally become swollen and hyperæmic, and numerous hæmorrhages occur in the omentum, mesentery, and serosa of the gut and abdominal wall.

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[14] *Arc. f. exp. Pathologie u Pharmakologie*, 1878, viii, 361.

[15] *Deutsche med. Wochenschr.*, 1891, 976 and 1218.

[16] *Jour. Exp. Med.*, 1897, ii, 197.

TABLE IV.

| Series No. | Weight in grams | Amount of $HgCl_2$ | Death after — days | Result as regards the lesions of the cæcum |
|---|---|---|---|---|
| | | Controls | | |
| 29 | 810 | 0.038 | 3 | lesions of entire cæcum |
| 39 | 1295 | 0.06 | 3 | " " " |
| 44 | 1550 | 0.03 | 1 | no lesions |
| 36 | 1030 | 0.03 | 1 | " " |
| 57 | 1540 | 0.015<br>0.01 | 4 | lesions of entire cæcum |
| 60 | 1070 | 0.015<br>0.01 | 2 | " " " |
| | | Tied duct | | |
| 48 | 1350 | 0.03 | 7 | lesions confined to ileocæcal region |
| 50 | 1290 | 0.03 | 1 | 2 small areas of necrosis |
| 56 | 1310 | 0.015 | 1 | lesions confined to ileocæcal region |
| | | Permanent biliary fistula | | |
| 49 | 1320 | 0.03 | 1 | no lesions, except congestion |
| 55 | 1810 | 0.015;0.01 | 3 | mod. lesions of entire cæcum, becoming more intense at end of organ toward the appendix |
| 58 | | 0.015; 0.01 | 2 | slight lesions confined to ileocæcal region |

In Table IV are given the results of injections of corrosive sublimate into three series of rabbits: (1) into a control series, (2) into a series in which the bile duct was tied and severed, and (3) into a series in which biliary fistula had been produced. Only in the animals with tied bile duct could any lesions be found when death took place within twenty-four hours after the first injection of the mercury. All the animals which lived two days or longer showed lesions, but in the case of the animal with a tied duct which succumbed after seven days, they were slight, while in the two animals with fistulæ, one dying on the second and the other on the third day, the lesions were much less severe than in the corresponding controls. It would therefore appear that mercury, as is the case with dysentery toxin, is reabsorbed after secretion by the bile into the intestine and excreted chiefly by the cæcum and colon, which portion of the gut shows the severest grade of injury. As in the case of dysentery toxin, the process of elimination is a gradual one.

In order to test this view of the reabsorption of mercury by the bile from the small intestine enterostomy was performed on two rabbits. In the first the small intestine was attached to the abdominal wound at a point about two inches above the ileocæcal valve, after which the intestine was tied off above the valve. After closing the opening in the abdominal wall the small intestine was opened. There was free outward passage of contents. Two doses of sublimate of 0.02 gram each were given subcutaneously on October 17th and 18th. Rabbit dead on October 19th. Fistula opening patent; cæcum showed very few small lesions at the edges of the spiral valves; no lesions of the colon. In making the opening in the intestine of the second rabbit a small cannula was employed. October 16th, operation done; October 17th and 18th each 0.02 gram sublimate injected subcutaneously. Rabbit dead October 19th. The autopsy showed that the opening of the cannula was occluded by a mass of dried intestinal contents; so that no fluid could escape. The cæcum showed hæmorrhages into the spiral valve with superficial necrosis, and hæmorrhagic infiltration of the colon. This second experiment, through the accident of the closure of the cannula, becomes a convincing control for the first one. It may, therefore, be considered as rendered probable that the intestinal lesions in mercury poisoning are caused not necessarily by the mercury present originally in the blood, but by a process of successive elimination and reabsorption of the metal from the intestinal tract in which process the biliary secretion plays a highly important part.

In carrying out the experiments with ricin a sample made by Merck, the toxicity of which was about 0.1 milligram per kilo of rabbit, was employed. The control rabbit, weighing 1420 grams, was given 0.15 milligram of ricin intravenously on January 27th. Death occurred twenty-four hours later. At autopsy the characteristic lesions inclusive of increased fluid in the pericardium and peritoneum, hæmorrhages into the epicardium and omentum, swelling and hæmorrhage of the superficial and deep lymphatic glands, much swelling of Peyer's patches, increase of mucoid fluid in the small intestine, and enlargement ( ×2) of the spleen existed.

In a second rabbit, weighing 1470 grams, a permanent biliary fistula was made on January 26th, and immediately after the operation 0.15 milligram of ricin was injected. Death took place the next day. The autopsy showed slight swelling of and no hæmorrhage in the lymphatic glands, no excess of fluid in the pericardium and peritoneum, no hæmorrhages in the epicardium or peritoneum, slight swelling of Peyer's patches, and contracted (normal) condition of the small intestine. A repetition of this experiment, using twice the dose, was made. The control and operated rabbits were given 0.3 milligram per kilo of body weight. The former succumbed after nineteen, the latter after twenty-two hours. The lesions in the first were very pronounced and of a strikingly hæmorrhagic character. Considerable hæmorrhage had taken place into the mucosa and contents of the cæcum and appendix. In the fistulous rabbit the lesions were more marked than in the first operated animal and much less severe than in the control rabbit. While some of the peripheral lymphatic glands were not perceptibly enlarged, hæmorrhages had taken place into others, and still others were congested. The patches of Peyer were only slightly swollen; but a few small punctiform hæmorrhages occurred in them; the lymphatic tissue of the cæcum was swollen but free of hæmorrhages.

The experiments with ricin leave no doubt that the pathological effect of the poison is greatly modified by permitting the bile to escape from the intestine; and while the fatal outcome is, perhaps, only delayed by the operation, the profound difference in the tissue and vascular reactions indicate that an essential part or effective quantity of the poison leaves the body with the bile. But in addition the experiments prove that resorption of ricin takes place from the intestinal canal, and the lesions met with also point to an excretion by this organ.

The three sets of experiments which have been presented would seem to have important features in common. If it is permissible to apply the chief facts of them to the interpretation of the production and localization of diphtheritic dysenteric lesions in man, it may be said that these lesions are due to the action of a toxin elaborated by the dysentery bacilli present in the diseased intestine, which toxin is first absorbed, in the main probably from the small intestine, and eliminated chiefly through the large intestine, which suffers injury through the act of excretion. In offering this view of the pathogenesis of dysentery we would add that the injury upon the tissues once inflicted in the manner mentioned, other micro-organisms than the dysentery bacillus doubtless come into action and complicate and increase the pathological effects of the dysentery toxin.

A study of the histology of the lesions of experimental colitis tends to confirm the conclusion that the poison which causes degeneration, necrosis, and inflammation of the cæcum acts not first upon the surface of the mucous membrane, but upon the mucous membrane and the submucosa simultaneously. In the purely œdematous lesions the mucosa may entirely escape noticeable injury, even the surface epithelium being retained in normal appearance. It is, however, usual for the mucous membrane in these cases to show œdema also, especially of the layer next to the muscularis mucosæ. The exudate in these cases is an inflammatory œdema giving rise to fibrin formation in the submucosa; no striking number of polymorphonuclear leucocytes are seen in the tissues although in the veins they may be increased. The blood-vessels are usually congested.

When hæmorrhage is associated with the œdema the mucous membrane is much more altered. The extravasation of blood takes place chiefly in the mucous membrane and usually causes disintegration of the glandular tissue. Associated with the hæmorrhage is, often, necrosis of the cellular elements of the mucous membrane, the necrosis exhibiting itself by hyaline degeneration of the glandular cells, fragmentation, etc. In the cases in which there is much disintegration and necrosis of the mucous membrane, the submucosa shows, besides œdematous swelling, an invasion of polymorphonuclear cells, and thrombosis of lymph-vessels and of the dilated veins. There is no sharp line between this condition and the one in which the mucous membrane shows coagulative necrosis and thrombi, and the submucosa an abscess-like condition from a great accumulation of leucocytes. Whenever the submucosa is markedly necrotic it is more or less densely infiltrated with leucocytes, which show, moreover, a decided tendency to collect in two layers—one being in relation to the muscularis mucosæ, the other in relation to the circular muscle. Both of these structures may be invaded with leucocytes. On the other hand, the submucosa may show so great an accumulation of leucocytes of the polymorphonuclear variety that the appearance suggests the formation of an abscess and yet the mucous membrane may be intact. The leucocytes show advanced necrotic changes, but the intercellular tissue does not undergo softening. A true abscess is, therefore, not formed.

The tendency of the pathological changes in the mucosa and submucosa to appear with greatest intensity in the spiral folds is clearly seen in the microscopical sections. The lesions diminish in intensity as the level surface of the mucosa is reached. While the entire depth of the mucosa in the full length of the fold may show coagulative necrosis, or hæmorrhage with disintegration or œdema, as the lower margins are approached the lesions extend less in depth and become discontinuous, until at the level of the mucosa microscopical areas, chiefly superficial ones, of necrosis or hæmorrhage appear in the mucosa, and the submucous infiltration is disappearing. On the surface and within the substance of the necrotic mucous membrane a definite fibrinous and leucocytic pseudo-membrane may form. In this and in the adjacent necrotic membrane a massive bacterial development may take place.

While it is not our intention to describe the histological character of mercury colitis it is proper to state that a study of these lesions has shown us that they differ from those caused by dysentery toxin only in intensity. In localization and in character they resemble the severer type of lesions just described.

## SUMMARY.

1. The facts described in this paper are intended to bear upon the pathogenesis of colitis in man and animals.

2. The toxin of the Shiga dysentery bacillus is liberated from the bacillus through the process of autolysis.

3. In rabbits the toxin is not absorbed directly in an active form by the gastro-intestinal tract; in man, however, absorption of an active poison does take place from the intestine.

4. The toxin is excreted in rabbits, and probably in man as well by the intestine, chiefly probably by the large intestine, which being injured by the act of elimination, reacts by the development of inflammation, etc.

5. In rabbits the characteristic action of the toxin depends upon the integrity of the biliary secretion into the intestine. When the bile is prevented from entering the intestine, either by ligature and section of the duct or by establishment of a biliary fistula, no lesions whatever of the large intestine appear, or they are inconsiderable in extent.

6. The loss of toxin through a biliary fistula does not prevent in rabbits the lethal effects which are caused, apparently, by a nervous poison. The tying off of the bile duct seems to prevent the passage of the toxin, in amounts sufficient to cause the large intestinal lesions, into the blood. The liver, therefore, in this condition tends to hold back the toxin from the general circulation.

7. The peculiar effects of the toxin on the large intestine in rabbits is not produced at once, but would appear to depend upon successive acts of excretion of the poison by the bowel.

8. The establishment of biliary fistula reduces the intensity of action of corrosive sublimate upon the large intestine in rabbits; and the lesions of ricin poisoning in these animals are also modified by this operation.

9. Dysentery toxin is destroyed by peptic digestion, and also, though probably more slowly, by tryptic digestion. The absence of power of the toxin to cause poisoning in rabbits when it is brought directly into the lumen of the intestine, is not explained by the destructive action of trypsin.

10. The character of the histological changes in the cæcum of rabbits caused by the dysentery toxin points to an action upon the substance and not primarily upon the surface of the intestine.

# SOLUTION OF TISSUE WITH ABSCESS.

By EUGENE L. OPIE, M.D.

(*From the Rockefeller Institute for Medical Research, New York.*)

In a former publication[1] I have shown that the two types of cells which especially act as phagocytes, namely the polynuclear leucocytes with fine granulation and the large mononuclear phagocyte or macrophage of Metschnikoff, are each characterized by a distinct proteolytic enzyme. That enzyme which is contained in the polynuclear leucocytes and as I have previously shown[2] is manufactured in the bone marrow is capable of causing proteolytic digestion in the presence of a neutral or alkaline reaction and is almost wholly incapable of action when placed in an acid medium. For this enzyme I have suggested the name leuco-protease, since it is characteristic of leucocytes contained in pus and other inflammatory exudates. The large mononuclear phagocytes of inflammatory exudates are especially concerned with the ingestion and destruction of cellular elements; the same cells are found in lymphatic glands adjacent to the site of inflammation and are doubtless identical with those which, for example, are abundant in the spleen lymphatic glands and other situations with typhoid fever. These cells contain an enzyme which is incapable of digesting proteid in an alkaline medium but is active in the presence of a weak acid; I have suggested for this enzyme the name lympho-protease.

The serum of an exudate produced by injecting aleuronat into the pleural cavity of a dog inhibits the proteolytic activity of leuco-protease[3]. The serum of the blood has the same property. Since this enzyme contained in the polynuclear leucocytes is incapable of digesting proteid in the presence of the blood serum its action is limited to these cells;

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[1] *Jour. Exper. Med.*, 1906, viii, 410.

[2] *Ibid.*, 1905, vii, 759.

[3] *Ibid.*, 1905, vii, 316.

it can act only upon substances which have been ingested by these phagocytes and thus removed from the influence of the serum. Doubtless the same property of the serum serves to protect the tissues from the proteolytic enzyme contained in the polynuclear leucocytes, for even should it be set free by disintegration of these cells or otherwise its activity would be inhibited. Since lympho-protease does not cause proteolysis in a neutral or alkaline medium, the alkalinity of the blood serum would check its action should it be set free by disintegration of mononuclear phagocytes.

An experiment in which increasing quantities of serum are allowed to act upon the same quantity of enzyme demonstrates very clearly this anti-enzymotic property of the serum. Previous experiments have shown that the cells of an exudate obtained by injecting aleuronat into the pleural cavity of a dog preserve their power to cause proteolysis when dried after treatment with absolute alcohol and ether. Leuco-protease alone is preserved by this method. A weighed quantity of the dry enzyme has been allowed to act during five days at 37° C. upon a measured quantity of diluted blood serum (10 c.c.) heated to 75° C. for one half-hour and thus partially coagulated and denaturalized. The methods employed have been described in previous articles.

Nitrogen in substances uncoagulable by heat has been estimated by the Kjeldahl method and for convenience is represented by cubic centimeters of 1/10 N sulphuric acid.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 20 | mgr. powdered leucocytes | + | coagulated proteid | | | | 24.2 c.c. |
| 20 | " " " | + | " " | + | 0.25c.c. | serum | 22.15 " |
| 20 | " " " | + | " " | + | 0.5 " | " | 18.8 " |
| 20 | " " " | + | " " | + | 1.0 " | " | 10.6 " |
| 20 | " " " | + | " " | + | 2.5 " | " | 7.55 " |

Control, 2.85c.c.

Two and a half cubic centimeters of serum has almost completely inhibited the proteolytic action of twenty milligrams of the powdered cells (by subtracting nitrogen in uncoagulable form contained in the control mixture, digestion is found to be represented by 3.28 c.c.) while the addition of only a quarter of a cubic centimeter materially hinders digestion.

By adding increasing quantities of the enzyme-containing powder to the same quantity of serum it has been found that a given quantity of serum can inhibit the action of only a limited quantity of enzyme.

| | | | |
|---|---|---|---|
| 10 mgr. powdered leucocytes | + coagulated serum | + 2.5 c.c. serum | 6.15 c.c. |
| 20 " " " | + " " | + 2.5 " " | 6.5 " |
| 40 " " " | + " " | + 2.5 " " | 10.4 " |
| 80 " " " | + " " | + 2.5 " " | 19.2 " |
| 160 " " " | + " " | + 2.5 " " | 25.15 " |

Nitrogen in uncoagulable form in coagulated serum + 2.5 c.c. serum is represented by 6.0 c.c.; in 160 mgr. powdered leucocytes, by 2.4 c.c.

The enzymotic activity of the powder used in the preceding experiment is shown by the following test:

| | |
|---|---|
| 20 mgr. powdered leucocytes + coagulated serum | 18.9 c.c. |
| Control | 4.3 " |

Two and a half cubic centimeters of serum have completely inhibited twenty milligrams of the dried cells but have failed to prevent proteolysis when a larger quantity of enzyme is employed.

In a former publication I have shown that exudates removed from the pleural cavity of a dog one or two days after the injection of aleuronat fail to undergo autolysis because the anti-enzyme of the serum inhibits the proteolytic enzymes of the leucocytes. In exudates removed three days after injection of the inflammatory irritant slight autolysis occurs. Variation in the enzymotic activity of the serum of the exudates doubtless explains the inconstant results of Schutz[4] and of Zak,[5] who failed to find in human exudates any relation between the autolysis of an exudate and the abundance of its cellular elements.

Since an abscess is characterized by softening and solution of tissue it has seemed not improbable that the fluid obtained by centrifugalization from purulent exudates would fail to exhibit the anti-enzymotic action which is characteristic of sterile exudates produced in the pleural cavity by aleuronat, and, doubtless, according to the previously mentioned observations of Schutz and of Zak, of certain human exudates. As the result of the experimental pleurisy produced by aleuronat, at least, the chest wall is uninjured and the pleural cavity, save for a few adhesions, may return to a normal condition.

[4] *Cent. für inner. Med.*, 1902, xxiii, 1161.

[5] *Wiener klin. Woch.*, 1905, xviii, 376.

A sterile purulent exudate was obtained by injecting a small quantity (one cubic centimeter) of turpentine into the subcutaneous or intermuscular tissue of the flank of the dog. At the end of four or five days a large cavity distended with fairly thick purulent fluid is formed. Such purulent fluid contains polynuclear leucocytes in immense number together with mononuclear cells, fat globules, and particles of degenerated cells. Agar-agar inoculated with this purulent exudate has in every instance remained sterile. A measured quantity (5 c.c.) of this exudate was diluted with four times its volume of 0.85 per cent. sodium chloride and after addition of toluol subjected for five days to a temperature of 37° C.

| | |
|---|---|
| 5 c.c. pus at 37° C. for 5 days | 12.6 c.c. |
| Control | 7.05 " |
| Digestion | 5.55 " |

The following experiment demonstrates that the same purulent exudate is capable of digesting coagulated proteid under the conditions just mentioned.

| | |
|---|---|
| 2.5 c.c. pus + 5 c.c. coagulated serum | 17.45 c.c. |
| Control | 6.4 " |
| Digestion | 11.05 " |

In order to determine if the proteolysis caused by the purulent exudate is due to the absence of anti-enzyme in the serum of the exudate, cells were separated from the serum by centrifugalization. A cloudy fluid containing globules of fat but no leucocytes was obtained. The effect of this serum upon a given quantity of enzyme preserved as a dry powder was compared with the anti-enzymotic action of the blood serum from the same animal. In the following experiments the dry enzyme was prepared from the cells of a purulent exudate obtained after injecting turpentine into the subcutaneous tissue of a dog. Twenty milligrams of the powdered leucocytes were allowed to act upon coagulated serum in the presence of 2.5 c.c. of serum of the pus from which the dried enzyme was obtained or in the presence of serum of the blood of the same animal.

| | Serum of pus | Serum of blood |
|---|---|---|
| With 2.5 c.c. of serum | 14.15 c.c. | 5.15 c.c. |

The proteolysis produced by the same quantity of enzyme acting upon the same quantity of proteid without addition of serum is represented by 16.5 c.c.

The following experiment, in which the conditions do not differ from those just described save that the preserved enzyme was obtained from an exudate produced by injecting aleuronat into the pleural cavity, further demonstrates the almost total absence of anti-enzymotic action in the serum from the abscess.

| | Serum of pus | Serum of blood |
|---|---|---|
| With 1.5 c.c. of serum | 17.0 c.c. | 9.6 c.c. |
| With 2.5 c.c. of serum | 17.6 " | 9.4 " |

Proteolysis produced by the same quantity of enzyme alone is represented by 17.95 c.c., the control containing nitrogen in uncoagulable form represented by 3.95 c.c.; 2.5 c.c. of serum of pus contains nitrogen in uncoagulable form represented by 2.1 c.c.; 2.5 c.c. of blood serum, by 1.7 c.c.

A similar experiment in which serum from pus obtained five days after the injection of turpentine was used, confirms those just described. A measured quantity of coagulated proteid was subjected to the action of twenty milligrams of powdered leucocytes in the presence of one and of two and a half cubic centimeters of serum of pus or, for the sake of comparison, of blood.

| | Serum of pus | Serum of blood |
|---|---|---|
| With 1. c.c. of serum | 17.6 c.c. | 6.7 c.c. |
| With 2.5 c. c. of serum | 18.65 c.c. | 7.35 " |

Proteolysis produced by the same quantity of enzyme without the addition of serum is represented by 15.65 c.c., the control containing uncoagulable nitrogen represented by 4.3 c.c.; 2.5 c.c. of serum of pus contains nitrogen in uncoagulable form represented by 3.3 c.c., and the same amount of serum of blood by 1.7 c.c.

When allowance is made for the quantity of nitrogen in uncoagulable form in the various mixtures before digestion, it is evident that one cubic centimeter of blood serum completely inhibits the activity of twenty milligrams of the powdered leucocytes, but more than twice as much serum from the purulent exudate has no inhibitory action.

Since the purulent exudates used in the experiments just described have been obtained by injecting turpentine into the subcutaneous tissue, it is possible that the absence of anti-enzyme in the serum of these exudates may be due to the action of this substance. In the following experiment the ability of 2.5 c.c. of serum to inhibit the digestion of coagulated proteid caused by forty milligrams of powdered leucocytes was tested in the presence and in the absence of turpentine. For the sake of comparison the same amount of enzyme was allowed to act upon coagulated proteid with and without turpentine in the presence of 2.5 c.c. of blood serum heated to 75° C in order to destroy its anti-enzymotic activity:

| | Without turpentine | With turpentine |
|---|---|---|
| With 2.5 c.c. heated serum | 31.2 c.c. | 28.05 c.c. |
| With 2.5 c.c. serum | 5.55 " | 5.4 " |
| Control | 3.55 " | 4.3 " |

Leuco-protease is capable of causing proteolysis in the presence of turpentine and the power of the blood serum to inhibit this digestion is unaffected by turpentine.

It is not improbable that an increasing quantity of proteolytic enzyme set free by disintegration of polynuclear leucocytes has so far overcome the anti-enzymotic action of a limited quantity of exuded serum that the entire pus is capable of active autolysis and has the power of digesting foreign proteid. The limited ability of a given quantity of blood serum to inhibit the action of increasing quantities of enzyme, demonstrated by the experiment already described, gives confirmation of this view. A purulent exudate is characterized by its ability to dissolve fibrin and necrotic tissue because its serum, unlike that of the non-purulent exudate, does not check the activity of the proteolytic enzyme furnished in great abundance by the polynuclear leucocytes.

# A SIMPLE METHOD FOR THE PLATING OF ANAËROBIC ORGANISMS.

By HANS ZINSSER, M.D.,
*Assistant in Bacteriology.*

PLATE XXV.

*(From the Bacteriological Laboratory of the College of Physicians and Surgeons.)*

It is not without hesitation that the writer ventures to add another to the already long list of procedures for the separation of anaërobic bacteria. He is encouraged, however, to describe the method given below by the hope that it may prove useful to other workers in the same field by its extreme simplicity and ease of application, and by its economy of time, in that it makes possible the use of the usual plating method in anaërobic studies.

The most satisfactory methods employed heretofore for the separation of anaërobes have depended almost without exception upon the use of a hydrogen generator. This, with its accessory solutions for the removal of impurities derived from the zinc (lead acetate and silver nitrate), is a formidable apparatus, difficult to keep in order and complicated in application. It is hardly necessary to emphasize to those who have worked with anaërobic organisms the desirability of eliminating the necessity for the use of hydrogen.

The most efficient of the methods of anaërobic separation without hydrogen is that of Roux, who used ordinary glass tubes pointed at the ends. The inoculated agar is drawn into these, the ends are sealed in a flame, and practically anaërobic conditions secured. When colonies have developed, the glass is cracked; or, even better, as the writer has found, the entire agar cylinder is pushed out into a sterile Petri dish with a glass rod of suitably small caliber. The method of Roux, while simple and efficient, presents a number of practical disadvantages. The mass of agar used is of necessity very small and unless, therefore, the dilution of organisms has been extreme, the colonies will be very close together. Again, the convexity of the surface of the agar cylinder renders microscopical study of the colonies very unsatisfactory. The last but chief objection to this method is that there is a considerable absorption of oxygen by the agar during the time in which it is cooling sufficiently to permit its inoculation. While this, of course, is insufficient to prevent the growth of anaërobic organisms, it is enough to mar any conclusion that could be drawn as to the behavior of such organisms under absolutely oxygen-free environments.

The method to be described, the writer believes, besides being a facile one for separation, permits the assumption of absolute anaërobiosis. It is due entirely to the suggestion of Prof. Hiss, who proposed to the writer the simplification for plating purposes of the usual pyrogallic acid methods, that a successful method was devised.

The apparatus used consists of two circular glass dishes, fitting one into the other as do the halves of a Petri dish, and similar to these in every respect except that they are higher, and that a slightly greater space is left between their sides when they are placed together. The dishes should be about three-fourths to one inch in height, they need be of no particular diameter although those of about the same size as the usual Petri dishes are most convenient. The sole requirement necessary for successful plating is that the trough left between the two plates when put together shall not be too broad, a quarter of an inch being most favorable.

Into the smaller of these plates the inoculated agar is poured exactly as is done into a Petri dish in the ordinary anaërobic work. Prolonged boiling of the agar before plating is not essential. When the agar film has become sufficiently hard on the bottom of the smaller dish, the entire apparatus is inverted. The smaller dish is now lifted out of the larger, and placed, still inverted, over a moist surface—a towel or the wet surface of the table—to prevent contamination. Into the bottom of the larger dish, which now stands open, there is placed a quantity (one to two drachms) of dry pyrogallic acid. Into this, over the pyrogallic acid, the smaller dish, still inverted, is then placed. A strong solution of sodium hydrate is poured into the space left between the sides of the two dishes, in quantity sufficient to fill the

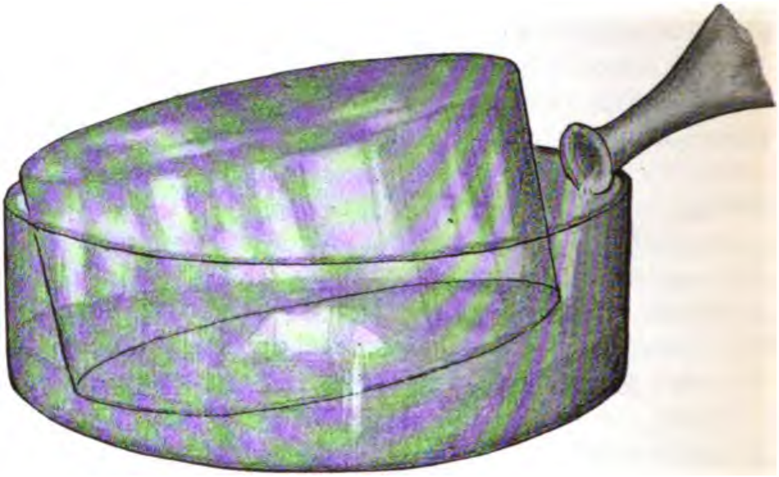

FIG. 1

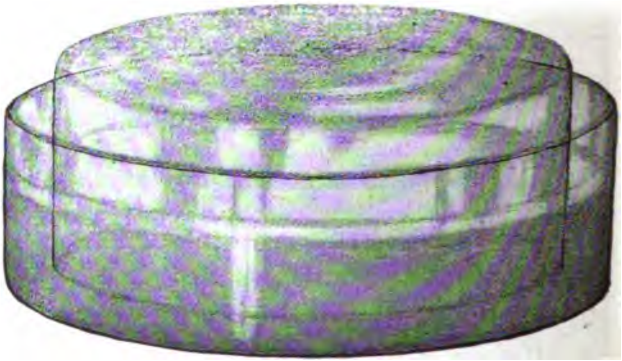

FIG. 2

receiving one half full. While this is gradually dissolving the pyrogallic acid (and this is the only step which requires speed) alboline, or any other oil, is dropped from a pipette, previously filled and placed in readiness, into the same space, thus completely sealing the chamber formed by the two dishes.

If these steps have been performed successfully, the pyrogallic solution will at this time appear of a light brown color, and the smaller plate, with its agar film, will float unsteadily above the other. Very rapidly, as the pyrogallic acid absorbs the free oxygen in the chamber, this plate is drawn down close to the other, and the acid assumes a darker hue, which remains without further deepening even after three or four days' incubation.

The accompanying photographs were made of cultures plated in this manner of B. tetani (Plate xxv, Fig. 1) and symptomatic anthrax ( Fig. 2 ). The tetanus culture was separated in this way from a mixed culture containing two other organisms. The colonies thus grown can be studied and fished from in the same way as other colonies grown in Petri dishes.

While the observations the writer has made with this method are limited to the study of three strains of B. tetani and one of the bacillus of symptomatic anthrax, and in both cases to a series not exceeding a dozen platings in each, a few peculiarities of growth have been so constant in their occurrence in all the plates that they are thought worthy of record, especially in the light of the recent work of Smith in America and Tarozzi in Italy, which tends to show that complete anaërobiosis is not the principal factor for the cultivation of the so-called anaërobic organisms, but rather secondary to the presence of the proper nutriment.

In all of the plates observed, the growth has been slow, colonies of the smallest visible size rarely appearing earlier than twenty-four to thirty-six hours in the case of tetanus, or eighteen to twenty-four hours in the case of symptomatic anthrax. These colonies have very rapidly reached their maximum, which in the case of tetanus was rarely larger than about one to two millimeters in size, either in plain agar or in the 1 per cent. glucose agar used. While the initial inhibition in the growth may have been due to oxygen still present in the agar, yet the colonies have remained extremely compact and have even after five or six days' incubation failed to grow more profusely. This appears rather to support the points made by Smith and Tarozzi, since with complete anaërobiosis and ordinary media a less luxurious growth was observed than that obtained by them with a less anaërobic environment but with more attention paid to the constitution of the media.

When fished and examined morphologically, after three or four days' incubation, many of the individuals of these colonies have, by the unevenness of their staining and variations of length, suggested degenerative change. In only two cases in the series observed were spores found in fishing from the colonies, and this in plates where, on account of slowness in the addition of the sealing covering of alboline, the pyrogallic solution was permitted to take on a deep black color (viz., saturation with oxygen). This latter point may in a measure account for the inability of the anaërobic bacteria to form spores under absolutely oxygen-free surroundings. And this again may tend to throw some light on the reason for the absence of the spores in some of the spore-forming anaërobic organisms in the circulation of infected subjects.

In conclusion, the writer takes pleasure in acknowledging his gratitude to Professor Philip Hanson Hiss, without whose suggestion these observations would hardly have been made.

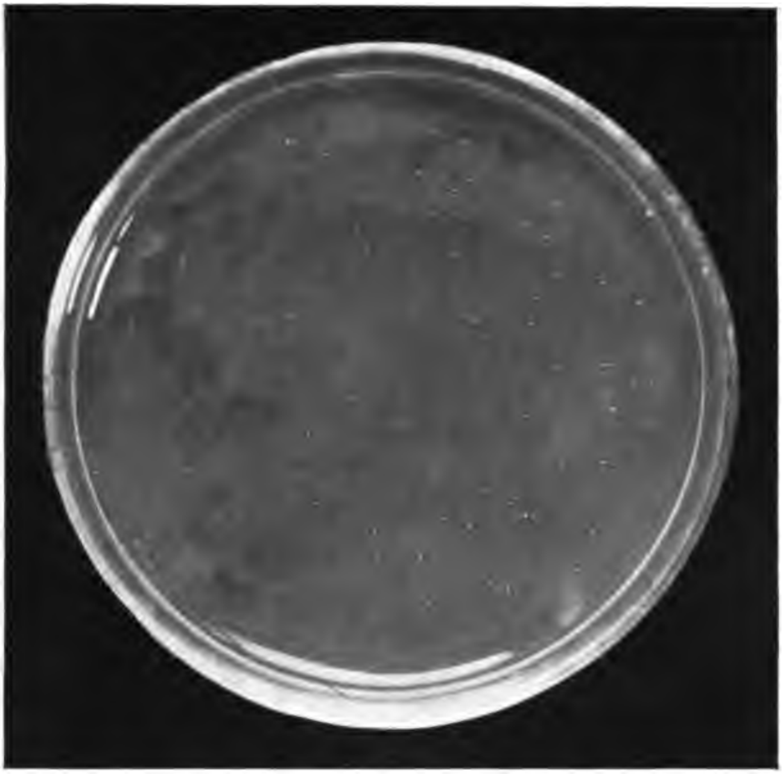

FIG. 1

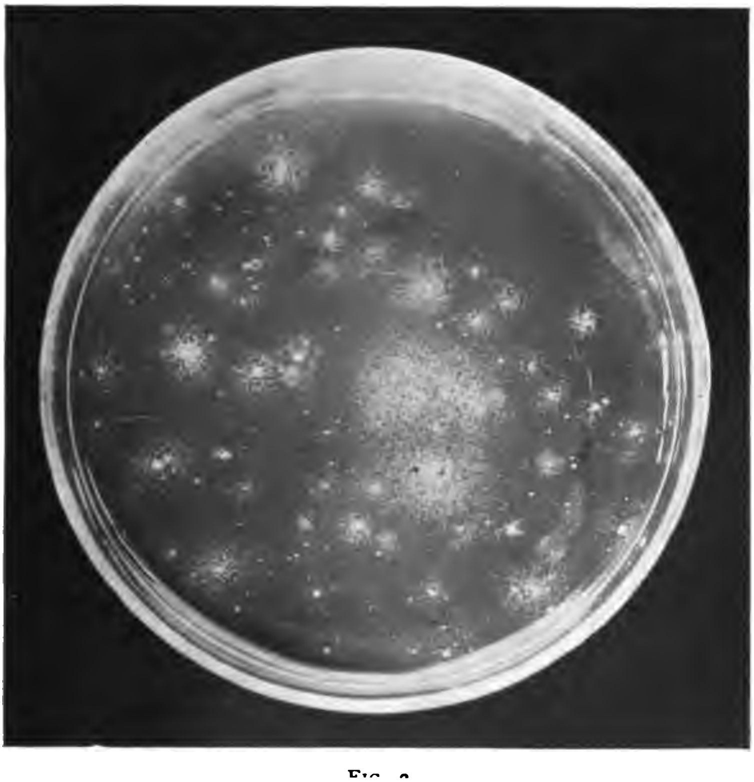

FIG. 2

# THE INFLUENCE OF COLLOIDS UPON THE DIFFUSION OF HÆMOLYSINS.

By SIMON FLEXNER, M.D., AND HIDEYO NOGUCHI, M.D.

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PLATES XXVI, XXVII, XXVIII, XXIX, AND XXX.

The study which forms the subject of this paper was suggested by the experiments made by one of us (Flexner[1]) upon the restraining influence of certain colloids upon the injurious action of bile salts upon the pancreas. The results of the present work not only confirm the view there expressed, namely, that colloidal substances reduce the degree and rapidity of diffusion of certain crystalloids, but they would seem to have even a wider biological bearing. It is for these reasons that they are given, in some detail, in this place. In order that the diffusion of very complex molecules (such as certain hæmolysins, etc.) might be studied it was necessary to devise a method of testing diffusion that would give approximately accurate results without having to resort to chemical analyses which are, indeed, useless with many of these bodies. We found that a suspension of blood corpuscles in certain colloids supplied a useful and accurate means of determining the rate of diffusion of simple and complex molecules which bring about hæmolysis. By reversing the experiment, namely by placing the chemical bodies in the colloids the rate of diffusion into water and saline could also be determined by estimating the quantity of the hæmolytic substance which appeared in these fluids after a given time.

The earlier studies on the influence of colloids upon the rate of diffusion showed, for the most part, that the rate remained unchanged. Graham[2] believed that no inhibition followed from the presence of colloids. He dissolved, among other things, 10 per cent. of sodium chloride in a 2 per cent. agar-agar jelly and overlaid this in a tube with another portion of the jelly minus the salt. At the end of eight days the amount of salt which diffused into the plain agar was found to be the same as that diffused into water. De Vries[3] failed to notice any marked difference in the diffusion of solutions of potassium chromate and copper sulphate into pure water and 4 per cent. silicic acid after a contact of four months. Voigtländer[4] investigated the rate of diffusion of certain simple acids and salts into agar-agar of from 1 to 4 per cent. While the rate of diffusion was at first in favor of the weaker concentration the actual quantities of salts and acids which diffused were about the same in all cases. Reformatsky[5] observed no inhibition of the catalytic reaction of acetate of methyl and hydrochloric acid by a 1.25 per cent. agar suspension; and Levi[6] ascertained that 1.57 per cent. silicic acid, 1 per cent. agar, and 0.6 per cent. gelatin did not alter electric conductivity, lower the freezing point, or inhibit the inversion of cane sugar by hydrochloric acid.

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[1] *Jour. Exp. Med.*, 1906, viii, 167.

[2] *Phil. Trans. Roy. Soc.*, 1861, cli, Part I, 183; *Liebig's Annalen*, 1862, cxxi, 1.

Not all experimenters have found that colloids act in a wholly indifferent manner upon the process of diffusion. Stefan[7] recalculated the values of Graham's experiments and ascertained that the diffusion constant was smaller with agar-agar jelly than with pure water. Chabry[8] found that gelatin and cartilage delayed the action of acids on orcein. While our own experiments were in progress a paper appeared by Kurt Mayer[9] in which the inhibiting action of gelatin, agar, and egg white upon diffusion is described. The bodies employed by him—sodium chloride and potassium chromate—were simple, and his results point to an unmistakable retardation of diffusion by colloids, the retardation being approximately proportional to the concentration of the colloids. With agar the differences are less marked than with gelatin, on account of the wider variations in concentration practicable with the latter substance. Similar results were obtained by Nell[10] who studied the effect of gelatin on the diffusion of a number of chemical bodies. Nell also observed that the electrical conductivity of solutions of copper sulphate are reduced by gelatin when present in amounts of from 1 to 20 per cent.

The number of substances the diffusion of which can be satisfactorily observed by the hæmolytic method is considerable. We studied acids, alkalies, sodium taurocholate, saponin, solanin, cobra venom, and tetanus toxin. In the case of the complex bodies, snake venom and tetanus toxin, the capacity for diffusion of the different constituents, namely, hæmolysins, neurotoxin, tetanospasmin, was also determined.

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[3] *Recueil des travaux chimiques de Pays-Bas*, 1884, iii, 375.

[4] *Zeitschr. f. physik. Chemie*, 1889, iii, 316.

[5] *Zeitschr. f. physik. Chemie*, 1891, vii, 34.

[6] *Gazetta chimica italiana*, 1900, xxx, Parte II, 64.

[7] *Wiener Sitzungsberichte*, 1879, lxxix, II Abt., 161 and 215.

[8] *Journal de Physique*, 1888, vii, 115.

[9] *Beiträge z. chem. Physiologie u. Pathologie*, 1905, vii, 393.

[10] *Annalen d. Physik*, 1905, IV Folge, xviii, 323.

The particular points which were noted were: the influence of concentration of colloids upon the rate of diffusion; the time relations of the rate of diffusion; and the comparative degree of diffusibility of different substances.

The diffusible bodies were made to diffuse (1) from saline solution into colloids, (2) from colloids into colloids, and (3) from colloids into saline solution.

### METHODS.

In preparing the various solutions and blood mixtures a fair degree of asepsis was observed. Where the experiments extended over any length of time greater precautions were taken. The blood was obtained aseptically and the colloids were sterile. The blood mixtures with agar and gelatin were well preserved at the end of a week or even longer. The experiments were made at room temperature which was about 20° C. The agar suspensions were made with agar-agar which had been thoroughly washed in running water. The agar was dissolved in distilled water, clarified, filtered, and 0.9 per cent. sodium chloride added. The gelatin was dissolved in distilled water, neutralized with NaOH, clarified, filtered, and salt in 0.9 per cent. added.

The highest concentrations of the colloids having been made, the weaker strengths were obtained by the addition of 0.9 per cent. salt solution. Acids, alkalies, and salts were made into normal solutions. These solutions were further diluted according to the hæmolytic strength of the different chemical bodies. In making the mixtures of chemical body and colloid a concentrated solution of the first was added to the second so as to avoid undue dilution of the colloid.

The colloids were pipetted into tubes of 8 mm. to 15mm. diameter, care being taken to prevent flowing over the wall of the tube above the layer. The quantities employed were, usually, 2 c.c. each of colloid and fluid. The colloid column measured from 32 to 33 mm. in tubes of 8 mm. diameter.

Dog and rabbit blood in 3 to 5 per cent. suspensions in 0.9 per cent. salt solution were used. The blood and colloid were mixed at low temperatures after which they were congealed. Ice was used to congeal the gelatin blood mixtures.

The readings were made with a scale one degree of which was 0.7 mm. All the figures were subsequently reduced to millimeters.

In determining the quantity of hæmolytic or toxic body which diffused from the colloid into the supernatant saline the latter fluid was removed at intervals and the hæmolytic or toxic strength determined. These values could be expressed in hæmolytic and in toxic units.

### DIFFUSION OF HÆMOLYSINS FROM FLUID INTO COLLOID MEDIA.

There is no difficulty in observing the extent to which the hæmolytic body has penetrated into the blood-colloid suspension since the originally opaque mixture becomes perfectly transparent as the result of the laking of the corpuscles. The line of penetration is usually sharp and straight, but in some cases it is irregular.

*Series I.*—Hydrochloric, nitric, oxalic acids, sodium hydroxide, and sodium carbonate in $\frac{1}{10}$ N. solutions were employed. The figures for hydrochloric and nitric acids are almost identical, although the degree of diffusion of hydrochloric is very slightly higher than of nitric acid. The values in millimeters for hydrochloric acid at the different periods (h. equalling hours) are as follows:

| | 9 h. | 18 h. | 42 h. | 60 h. | 84 h. |
|---|---|---|---|---|---|
| 25% gelatin | 4.5 | 7.7 | 13.0 | 16.5 | 18.2 |
| 10% " | 5.4 | 9.1 | 16.2 | 20. | 22.4 |

The values for oxalic acid are lower:

| | 9 h. | 18 h. | 42 h. | 60 h. | 84 h. |
|---|---|---|---|---|---|
| 25 % gelatin | 2.75 | 4.9 | 9.1 | 11.2 | 12.6 |
| 10 % " | 3.4 | 6.3 | 12.3 | 14.0 | 16.8 |

And the values for sodium carbonate are still lower:

| | 9 h. | 18 h. | 42 h. | 60 h. | 84 h. |
|---|---|---|---|---|---|
| 25 % gelatin | 1.25 | 2.24 | 4.2 | 4.9 | 5.6 |
| 10 % " | 1.75 | 3.3 | 6.5 | 8.4 | 9.8 |

The comparative rate of diffusion into gelatin of these simple bodies can be readily expressed graphically in the form of a curve (Plate XXVI). The influence of concentration of colloid on the rate and degree of diffusion is clearly seen in the curve. Lines I and II indicate the diffusion of hydrochloric acid into 10 per cent. and 25 per cent. gelatin respectively. Lines III and IV indicate nitric acid, V and VI oxalic acid, and VII and VIII sodium carbonate. The black dots show the reading periods and the marginal figures stand for millimeters.

On comparing the diffusion values for 10 and 25 per cent. gelatin the relation can be expressed by the following formula: *The amount of diffusion is approximately inversely proportional to the square root of the concentration of the gelatin.* The deviations from this formula can possibly be accounted for by considerations of experimental error, impurity in the gelatin, and the resistance introduced by the erythrocytes. The velocity of diffusion decreases steadily with increase in time.

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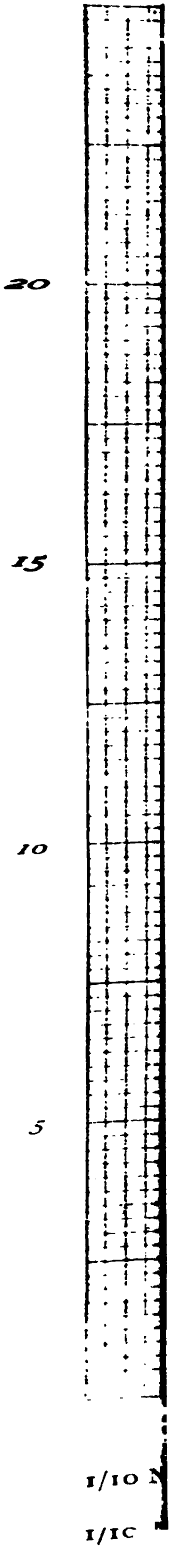

The concentration limits for agar are smaller than for gelatin and consequently the differences in degree and velocity of diffusion are also smaller with that colloid. The strengths employed in the next experiments were 0.5, 1, and 2 per cent. Two examples only, namely of nitric acid and sodium hydroxide, will be given.

$HNO_3$ $\frac{1}{10}$ N.

| | 15 min. | 30 min. | 1 h. | 2 h. | 3 h. | 4 h. |
|---|---|---|---|---|---|---|
| 2% agar | 5.6 | 9.5 | 13.5 | 18.5 | 21.3 | 24.5 |
| 1% " | 5.8 | 9.75 | 13.5 | 18.75 | 21.5 | 24.6 |
| 0.5% " | 5.95 | 9.8 | 13.8 | 19.0 | 21.8 | 24.8 |

NaOH $\frac{1}{10}$ N.

| | 15 min. | 30 min. | 1 h. | 2 h. | 3 h. | 4 h. |
|---|---|---|---|---|---|---|
| 2% agar | 5.6 | 9.5 | 13.5 | 18.5 | 21.3 | 24.5 |
| 1% " | 5.6 | 9.5 | 13.5 | 18.5 | 21.5 | 24.5 |
| 0.5% " | 5.95 | 9.6 | 13.75 | 18.75 | 21.5 | 28. |

*Series II.*—In the next experiments sodium taurocholate, saponin, and solanin were employed. The hæmolytic values were first determined in the ordinary manner. They can be expressed for rabbit blood in the following figures: 0.0001 (saponin), 0.0007 (solanin), 0.007 (sodium taurocholate). The value of hydrochloric acid, under the same conditions was 0.02.

Sodium Taurocholate $\frac{1}{10}$ N. Rabbit Blood 5%

| | 3 h. | 6 h. | 12 h. | 24 h. | 42 h. | 60 h. | 84 h. | |
|---|---|---|---|---|---|---|---|---|
| 25% gelatin | 1.0 | 2.0 | 3.15 | 4.3 | 5.6 | 7.0 | | 1st determination. |
| | | 1.2 | 2.2 | 3.9 | 5.6 | 7.0 | 8.4 | 2d " |
| 10% " | 1.4 | 2.6 | 4.3 | 6.6 | 7.8 | 9.0 | | 1st " |
| | | 2.0 | 3.5 | 5.6 | 7.7 | 9.3 | 11.2 | 2d " |

Saponin $\frac{1}{100}$ N. Rabbit Blood 5%

| | 3 h. | 6 h. | 24 h. | 48 h. | |
|---|---|---|---|---|---|
| 25% gelatin | 1.0 | 2.0 | 4.2 | 6.3 | 1st determination. |
| | | 2.4 | 5.0 | 7.0 | 2d " |
| 10% " | 1.4 | 2.9 | 6.6 | 9.15 | 1st " |
| | | 2.85 | 6.0 | 8.4 | 2d " |

Solanin $\frac{1}{100}$ N. Dog Blood 3%.

| | 6 h. | 24 h. | 48 h. |
|---|---|---|---|
| 25% gelatin | 3.6 | 7.5 | 10.5 |
| 10% " | 4.6 | 9.0 | 13.5 |

The relation which exists in the case of gelatin, between the degree of diffusion and the concentration of the colloid is, as this experiment shows, the same for certain chemicals of high and of low molecular weight. When agar-agar is substituted for gelatin the differences are less marked, although a difference can still be made out. Taking saponin as an example the following figures are obtained:

Saponin $\frac{1}{100}$ N.

| | 3 h. | 6 h. | 24 h. | 48 h. | |
|---|---|---|---|---|---|
| 1% agar | 2.45 | 5.0 | 9.8 | 14.0 | 1st determination. |
| 0.5% " | 3.85 | 8.4 | 17.5 | 24.5 | " " |
| 1% " | 1.7 | 3.5 | 8.9 | 10.5 | 2d " |
| 0.5% " | 1.8 | 3.8 | 9.3 | 11.2 | " " |

*Series III.*—The next experiments were made with the complex lysins of cobra venom and tetanus toxin. Two specimens of cobra venom were available: one a fresh solution of 0.4 per cent., one cubic centimeter of which contained for dog blood 2000 minimal hæmolytic doses; and a second solution of the same strength which had been on ice seven months and was ten times weaker. The samples of tetanolysin contained 33 minimal hæmolytic doses per cubic centimeter.

The experiments with dog blood and 0.1 per cent. cobra venom were not wholly satisfactory, since the line of demarkation was not always sharp. The influence of concentration of colloid is, however, shown even in this case.

| | 3 h. | 9 h. | 24 h. |
|---|---|---|---|
| 25% gelatin | 0.84 | 2.1 | 6.0 |
| 10% " | 2.1 | 5.6 | 31.5 |

The next experiment was made with cobra venom, lecithin, and rabbit corpuscles, in which case the diffusion is regular.

Cobra Venom 0.2% 1 c.c., Lecithin $\frac{1}{100}$ N. 1 c.c.

| | 12 h. | 24 h. | 42 h. | 60 h. | 84 h. |
|---|---|---|---|---|---|
| 25% gelatin | 1.7 | 2.8 | 4.20 | 4.9 | 5.6 |
| 10% " | 1.8 | 3.15 | 4.55 | 5.55 | 6.3 |

The employment of the old cobra venom gave, instead of a curve, an almost straight line.

Passing now to the results with cobra venom 0.1 per cent. and dog blood in agar, definite inhibition in the higher concentration is found to occur.

| | 6 h. | 24 h. | 48 h. |
|---|---|---|---|
| 2% agar | 1.4 | 5.25 | 9.1 |
| 0.5 " | 2.1 | 7.0 | 12.6 |

Cobra venom diffuses into agar more readily than into gelatin. The rate of diffusion into agar is proportional not to the square root of the time but almost directly to the time.

Tetanolysin penetrates gelatin (strength below 10 per cent.) more quickly than it does agar of 0.5 per cent., which makes an exception to the rule.*

### DIFFUSION OF HÆMOLYSINS FROM ONE COLLOID INTO ANOTHER.

The next experiments were designed to show whether any further inhibiting effect would be developed if the hæmolysin were enclosed in the colloid. Since the velocity of diffusion is smaller the higher the concentration of the colloid, it was presumable that the introduction of a second colloid medium would further reduce the velocity.

Sodium taurocholate, saponin, solanin, and pyrogallic acid were studied. In the first experiment to be given sodium taurocholate in $\frac{1}{10}$ N. solution was enclosed in gelatin of 10 and 25 per cent., and dog blood of 3 per cent. was suspended in agar of 0.5 and 2.0 per cent.

2% Agar and Dog Blood.

| Sod. taurocholate in | 6 h. | 12 h. | 24 h. | 48 h. | 66 h. |
|---|---|---|---|---|---|
| 25% gelatin | 1.55 | 2.4 | 3.15 | 4.0 | 5.6 |
| 10% " | 1.7 | 2.6 | 3.6 | 4.9 | 7.0 |
| Saline (control) | 2.55 | 3.55 | 5.15 | 7.3 | 9.8 |

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* Arrhenius and Madsen (*Contributions from the University Laboratory for Medical Bacteriology*, Copenhagen, 1902) have applied the method of diffusion into gelatin to a determination of the molecular weight of diphtheria toxin. They ascertained that diphtheria and tetanus toxin diffused into 10 per cent. gelatin; that the former underwent no change in the process and the latter diffused more rapidly than the former. The corresponding antitoxins passed into the gelatin more slowly than the toxins.

Craw (*Proc. of the Royal Soc.*, Series B., 1906, lxxvii, 311) determined that bacillus megatherium lysin passes more slowly through a porous filter impregnated with 15 per cent. than with 7.5 per cent. gelatin.

0.5% Agar and Dog Blood.

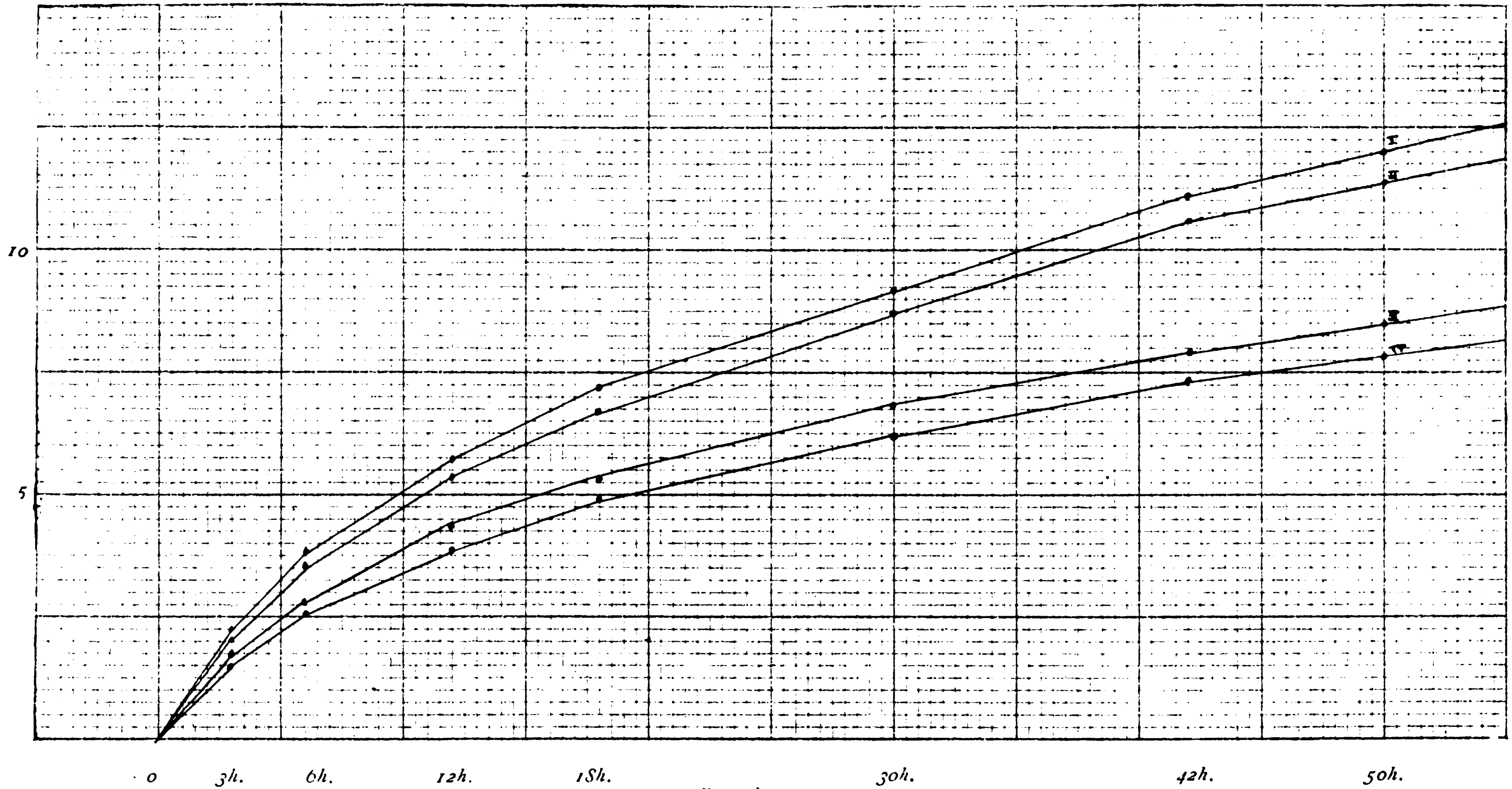
| Sod. taurocholate in | 6 h | 12 h. | 24 h. | 48 h. | 66 h. |
|---|---|---|---|---|---|
| 25% gelatin | 1.5 | 2.45 | 3.15 | 4.55 | 5.6 |
| 10% " | 1.8 | 2.8 | 3.8 | 5.0 | 7.0 |
| Saline (control) | 2.6 | 3.85 | 5.2 | 7.3 | 9.8 |

Hence taurocholate of sodium diffuses from 25 per cent. gelatin into agar more slowly than it does from 10 per cent. gelatin. The difference in concentration of the agar has only a slight influence on the rate of diffusion. In a second experiment the salt was dissolved in agar and the blood suspended in gelatin. The influence of concentration (0.5 and 2.0 per cent.) of the agar was very small, and the diffusion from agar was slightly greater than from saline solution (control).

The experiments with $\frac{1}{100}$ N. saponin dissolved in agar and dog blood suspended in gelatin showed diffusion to take place more rapidly into 10 than into 25 per cent. gelatin, and much more rapidly from agar than from the saline control. When, however, the colloids were reversed, namely, the blood suspended in agar and the solanin desolved in gelatin, the influence of the concentration of the gelatin came out, the diffusion from the saline being greater than that from the colloid. Plates XXVII and XXVIII express these effects in the form of curves.

The explanation of this discrepancy is to be found, possibly, in the sum of inhibitions exerted by the gelatin and the corpuscles, of which the first is the greater; while, as has frequently appeared in these experiments, the greatly weaker agar concentrations exercise slight inhibition only. A similar effect, although a smaller one, is produced in the case of pyrogallic acid.

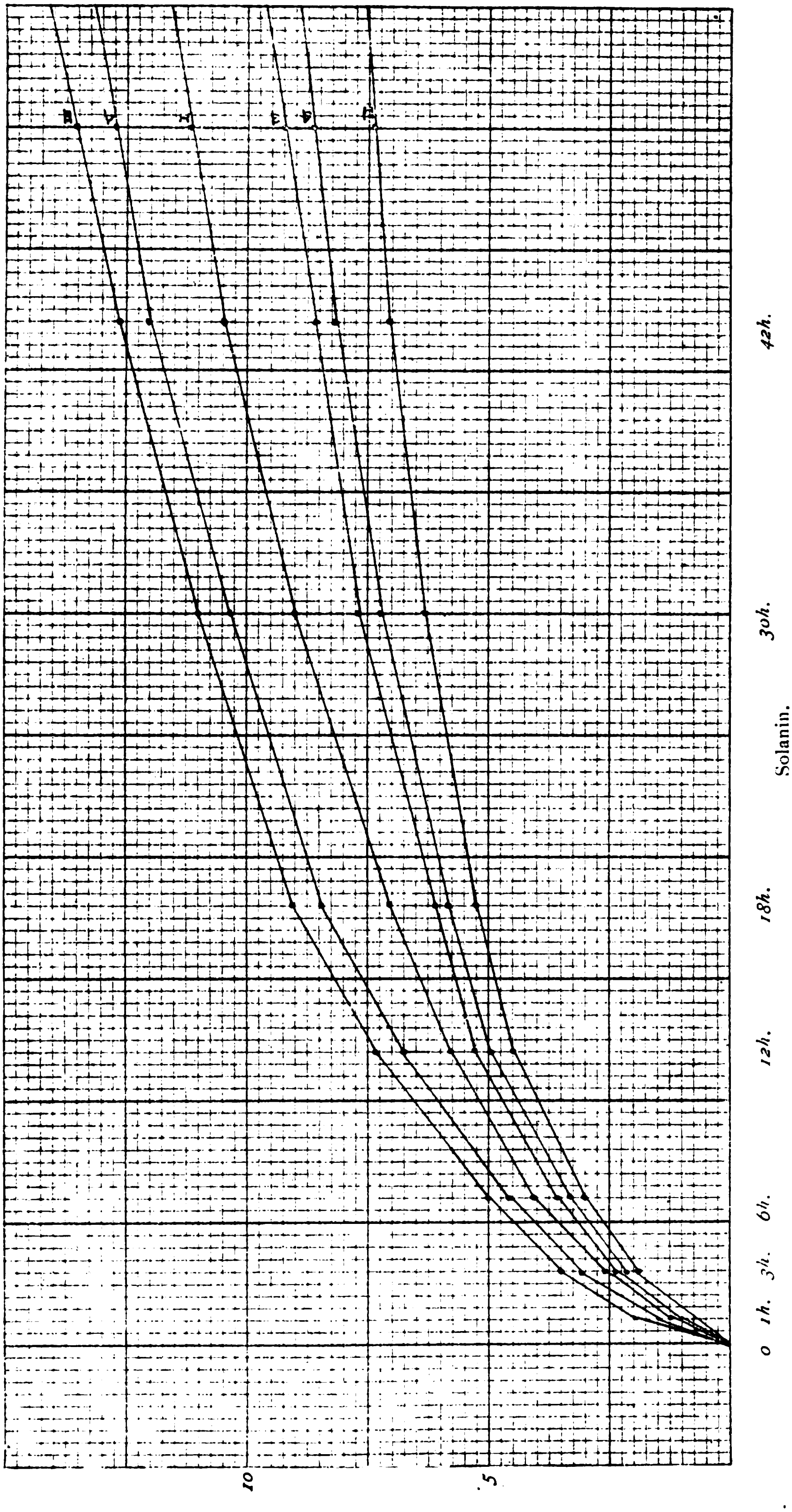
In the study of the more complex hæmolysins of venom it was found, in spite of certain irregularities of diffusion, that (1) the velocity of diffusion from agar into the gelatin blood is slower in the case of 25 than in the case of 10 per cent. gelatin, (2) the hæmolysin of cobra venom is diffused from agar into gelatine more slowly than from these colloids into saline, (3) concentrations of agar between 0.5 and 2.0 per cent. act about the same, and (4) the relation of time to diffusion does not correspond to Fick's law.



Saponin.

I From saline into 0.5% agar.
II From saline into 1% agar.

III From 10% gelatin into 1% agar.
IV From 25% gelatin into 1% agar.



Solanin.

I From saline into 10% gelatin.
II From saline into 25% gelatin.
III From 0.5% agar into 10% gelatin.
IV From 0.5% agar into 25% gelatin.
V From 1% agar into 10% gelatin.
VI From 1% agar into 25% gelatin.

## DIFFUSION FROM COLLOID INTO SALINE SOLUTION.

Sodium taurocholate, saponin, and solanin were dissolved in 10 and 25 per cent. gelatin and poured into test-tubes of 20 mm. diameter. Five cubic centimeters of the colloids were put into each tube after the congealation of which an equal quantity of saline solution was poured in. The saline fluid, after contact, was removed at stated periods, and the quantity of hæmolysin present in it was estimated colorimetrically. Three

### TABLE I.

SAPONIN $\frac{1}{100}$ N.

| Period of Contact | Degree of Hæmolysis | 25% gelatin | | 10% gelatin | |
|---|---|---|---|---|---|
| | | Quantity in c.c. required to produce stated degree of hæmolysis | Corresponding number of hæmolytic units in 1 c.c. of saline | Quantity in c.c. required to produce stated degree of hæmolysis | Corresponding number of hæmolytic units in 1 c.c. of saline |
| 1 hour | 100% | | | | |
| | 30% | 1.8 | 0.55 | 1. | 1. |
| | 5% | 0.9 | 1.1 | 0.55 | 1.8 |
| 2 hours | 100% | | | | |
| | 30% | 1.4 | 0.7 | 0.6 | 1.6 |
| | 5% | 0.7 | 1.4 | 0.5 | 2. |
| 4 hours | 100% | | | 1. | 1. |
| | 30% | 0.75 | 1.33 | 0.5 | 2. |
| | 5% | 0.5 | 2. | 0.35 | 2.8 |
| 24 hours | 100% | 0.7 | 1.4 | 0.5 | 2. |
| | 30% | 0.3 | 3.3 | 0.2 | 5. |
| | 5% | 0.18 | 5.5 | 0.14 | 7.1 |

SOLANIN $\frac{1}{100}$ N.

| | | | | | |
|---|---|---|---|---|---|
| 1 hour | 100% | 0.55 | 1.8 | 0.33 | 3. |
| | 30% | 0.14 | 7. | 0.09 | 11. |
| | 5% | 0.125 | 8. | 0.085 | 12. |
| 2 hours | 100% | 0.33 | 3. | 0.25 | 4. |
| | 30% | 0.09 | 11. | 0.075 | 13.3 |
| | 5% | 0.085 | 12. | 0.07 | 14. |
| 4 hours | 100% | 0.25 | 4. | 0.18 | 5.5 |
| | 30% | 0.07 | 14. | 0.055 | 18. |
| | 5% | 0.055 | 18. | 0.04 | 25. |
| 24 hours | 100% | 0.1 | 10. | 0.07 | 14.2 |
| | 30% | 0.03 | 33. | 0.025 | 40. |
| | 5% | 0.025 | 40. | 0.02 | 50. |

grades were used: 100, 30, and 5 per cent. It was found that sodium taurocholate was largely held back for at least 48 hours by the gelatin, probably because of a chemical union between the salt and the proteid. Only a trace of hæmolysis was obtained at the end of this period. The results obtained with saponin and solanin are given in the accompanying table (Table I), and they show that the quantities of these bodies diffused from 25 and 10 per cent. gelatin bear the proportion of 1 : 1.25–1.40. *Hence the velocity and amount of diffusion from colloid into saline, like that from saline into colloid, is inversely proportional to the square root of the concentration of the colloid.*

INFLUENCE UPON THE RATE OF DIFFUSION OF DIAMETER OF SURFACE, DEPTH OF MEDIUM, AND CONCENTRATION OF HÆMOLYSIN.

TABLE II.

| Diameter of Tube | Amount of |  | Period of Contact | $\frac{1}{100}$ N. Saponin |  | $\frac{1}{1000}$ N. Saponin |  | $\frac{1}{10000}$ N. Saponin |  |
|---|---|---|---|---|---|---|---|---|---|
|  | Gelatin Blood | Saponin Solution |  | 25% gelatin | 10% gelatin | 25% gelatin | 10% gelatin | 25% gelatin | 10% gelatin |
| 8 mm. | 4c.c | 4c.c. | 6 hours | 2.4 | 2.85 |  | 1.9 |  |  |
|  |  |  | 24 " | 5. | 6. | 4.2 | 4.55 | 1.75 | 1.75 |
|  |  |  | 48 " | 7. | 8.4 | 6.45 | 7.35 | 3.85 | 3.85 |
|  | 4 c.c. | 2 c.c. | 6 hours | 2.4 | 2.85 |  | 1.9 |  |  |
|  |  |  | 24 " | 5. | 6. | 4.2 | 4.55 | 1.75 | 1.75 |
|  |  |  | 48 " | 7. | 8.4 | 6.45 | 7.35 | 3.85 | 3.85 |
|  | 2 c.c. | 4 c.c. | 6 hours | 2.4 | 2.85 |  | 1.9 |  |  |
|  |  |  | 24 " | 5. | 6. | 4.2 | 4.55 | 1.75 | 1.75 |
|  |  |  | 48 " | 7. | 8.4 | 6.45 | 7.35 | 3.85 | 3.85 |
| 15 mm. | 2 c.c. | 2 c.c. | 6 hours | 2.4 | 2.85 | > |  |  |  |
|  |  |  | 24 " | 5. | 6. | 4.2 | 4.55 | 2. | 2.1 |
|  |  |  | 48 " | 7. | 8.75 | 7. | 7.7 | 3.85 | 3.85 |
|  | 2 c.c. | 2 c.c. | 6 hours | 2.4 | 2.85 | > |  |  |  |
|  |  |  | 24 " | 5. | 6. | 4.2 | 4.55 | 2. | 2.1 |
|  |  |  | 48 " | 7. | 8.75 | 7. | 7.7 | 3.85 | 3.85 |
|  | 1 c.c. | 2 c.c. | 6 hours | 2.4 | 2.85 | > |  |  |  |
|  |  |  | 24 " | 5. | 6. | 4.2 | 4.55 | 2. | 2.1 |
|  |  |  | 48 " | 7.5 | 9.1 | 7. | 7.7 | 3.85 | 3.85 |

The substances used in these experiments were solanin and saponin in saline solution and dog blood mixed with gelatin. The results are given in Tables II and III and they show that no marked influence is exercised on the diffusion by diameter of surface and depth of medium. But other facts may be noted.

TABLE III.

| Diameter of Tube | Amount of |  | Period of Contact | $\frac{1}{10}$ N. Solanin |  | $\frac{1}{100}$ N. Solanin |  | $\frac{1}{1000}$ N. Solanin |  |
|---|---|---|---|---|---|---|---|---|---|
|  | Gelatin Blood | Solanin Solution |  | 25% gelatin | 10% gelatin | 25% gelatin | 10% gelatin | 25% gelatin | 10% gelatin |
| 15 mm. | 4 c.c. | 4 c.c. | 6 hours | 3.6 | 4.6 |  | 1.25 | Slight trace in 48 hours. | ditto. |
|  |  |  | 24 " | 7.5 | 9.5 | 1.4 | 3.85 |  |  |
|  |  |  | 48 " | 10.5 | 13.5 | 2.1 | 5.95 |  |  |
|  | 4 c.c. | 2 c.c. | 6 hours | 3.6 | 4.6 |  | 1.25 |  |  |
|  |  |  | 24 " | 7.5 | 9.5 | 1.3 | 3.85 |  |  |
|  |  |  | 48 " | 10.5 | 13.5 | 2.1 | 6. |  |  |
|  | 2 c.c. | 4 c.c. | 6 hours | 3.6 | 4.6 |  | 1.25 |  |  |
|  |  |  | 24 " | 7.5 | 9.5 | 1.4 | 3.85 |  |  |
|  |  |  | 48 " | 10.5 | 13.5 | 2.1 | 5.95 |  |  |
| 8 mm. | 2 c.c. | 2 c.c. | 6 hours | 3.6 | 4.6 |  | 1.25 | Slight trace in 48 hours. | ditto. |
|  |  |  | 24 " | 7.5 | 9.8 | 1.4 | 3.85 |  |  |
|  |  |  | 48 " | 10.5 | 13.5 | 2.1 | 6. |  |  |
|  | 2 c.c. | 1 c.c. | 6 hours | 3.6 | 4.6 |  | 1.25 |  |  |
|  |  |  | 24 " | 7.5 | 9.8 | 1.4 | 3.85 |  |  |
|  |  |  | 48 " | 10.5 | 13.5 | 2.1 | 5.9 |  |  |
|  | 1 c.c. | 2 c.c. | 6 hours | 3.6 | 4.6 |  | 1.25 |  |  |
|  |  |  | 24 " | 8. | 9.8 | 1.4 | 3.85 |  |  |
|  |  |  | 48 " | 10.5 | 13.5 | 2.1 | 6. |  |  |

The relative capacity for diffusion of saponin and solanin from saline solution into gelatin blood is expressed in hæmolytic units by the proportion of 1:1.5. These figures are to be compared with those in which the hæmolysin is enclosed in the colloid (Table I) and allowed to diffuse into saline, in which series the solanin is shown to diffuse from seven to eight times more hæmolytic units in a given period than the saponin. But as solanin is nearly twice as strong an hæmolytic agent as saponin the proportion of diffused hæmolytic units would stand, as regards diffusion from colloid into saline, as 1:4 against 1:1.5 for saline into colloid. The diffusion velocity for solanin is, under the former conditions, four times as great as for saponin; and the inhibiting influence of the colloid is greater when the crystalloid seeks to enter it from than when it attempts to leave it for a fluid medium.

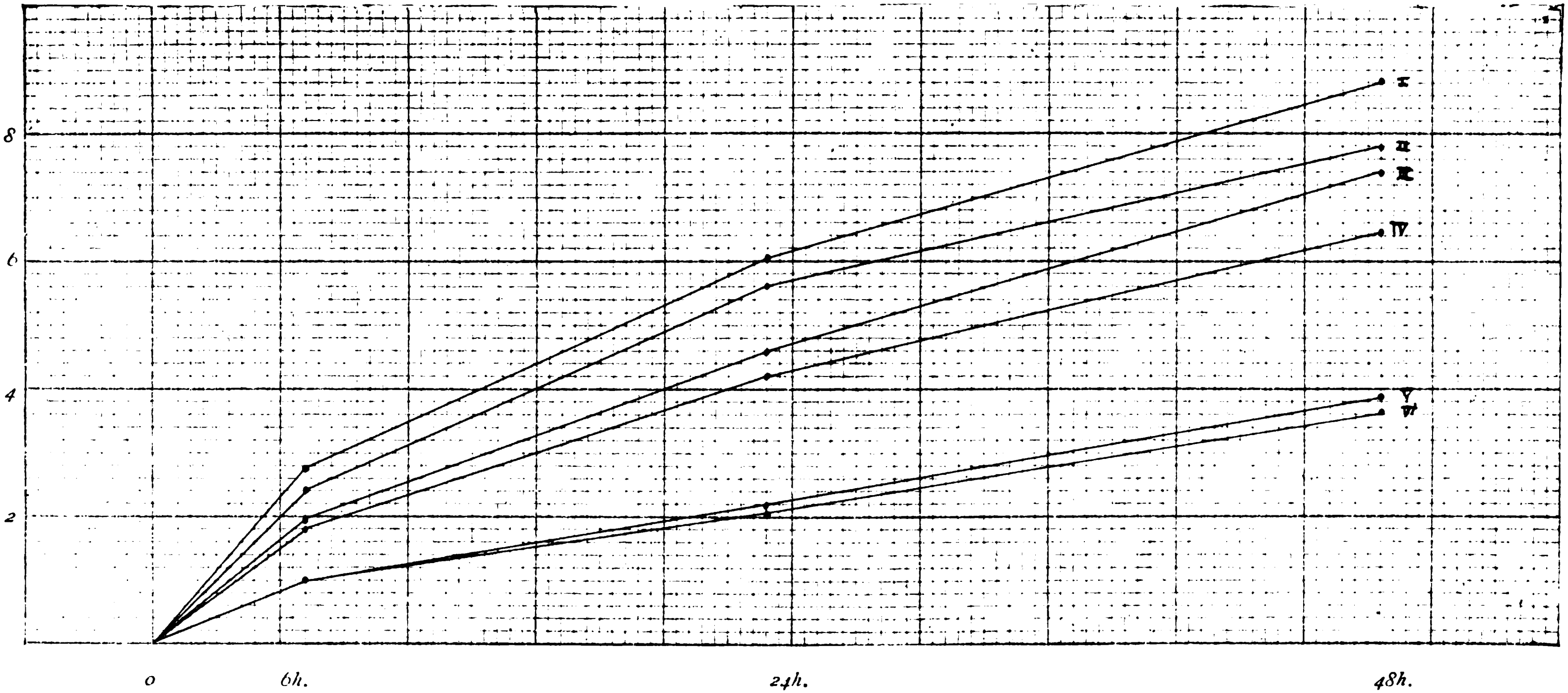
The concentration of the active substance plays a considerable part in relation to the rate of diffusion. The effects of this concentration are best seen by observing Plates XXIX and XXX. In the case of saponin while the difference in diffusion between $\frac{1}{100}$ N. and $\frac{1}{1000}$ N. is very slight, that between $\frac{1}{1000}$ N. and $\frac{1}{10000}$ N. becomes as about 2:1. In the case of solanin the difference between $\frac{1}{100}$ N. and $\frac{1}{1000}$ N. is as 2:1, and between $\frac{1}{1000}$ N. and $\frac{1}{10000}$ N. more than 10:1.

In Plate XXIX, the following values are given: I and II represent the degree of diffusion of $\frac{1}{100}$ N. saponin from saline into 10 per cent. and 25 per cent. gelatin respectively; III and IV represent the degree of diffusion of $\frac{1}{1000}$ N., and V and VI, the degree of diffusion of $\frac{1}{10000}$ N. saponin under the same conditions. The values for Plate XXX are as follows: I and II represent the degree of diffusion of $\frac{1}{100}$ N. solanin from saline into 10 per cent. and 25 per cent. gelatin respectively; and III and IV the degree of diffusion of $\frac{1}{1000}$ N. solanin under the same conditions.

TABLE IV.

| Scale of Hæmolysis* | Saponin | | | | Solanin | | | |
|---|---|---|---|---|---|---|---|---|
| | 25% gelatin | | 10% gelatin | | 25% gelatin | | 10% gelatin | |
| | $\frac{1}{100}$ N | $\frac{1}{1000}$ N | $\frac{1}{100}$ N | $\frac{1}{1000}$ N | $\frac{1}{100}$ N | $\frac{1}{1000}$ N | $\frac{1}{100}$ N | $\frac{1}{1000}$ N |
| a | 1. | | 0.6 | | 0.25 | 2. | 0.16 | 1.5 |
| b | 0.3 | | 0.25 | | 0.06 | 0.7 | 0.05 | 0.5 |
| c | 0.2 | 2. | 0.15 | 1.5 | 0.045 | 0.5 | 0.035 | 0.35 |

* The scale of hæmolysis was an arbitrary and descending one—a being highest and c lowest.

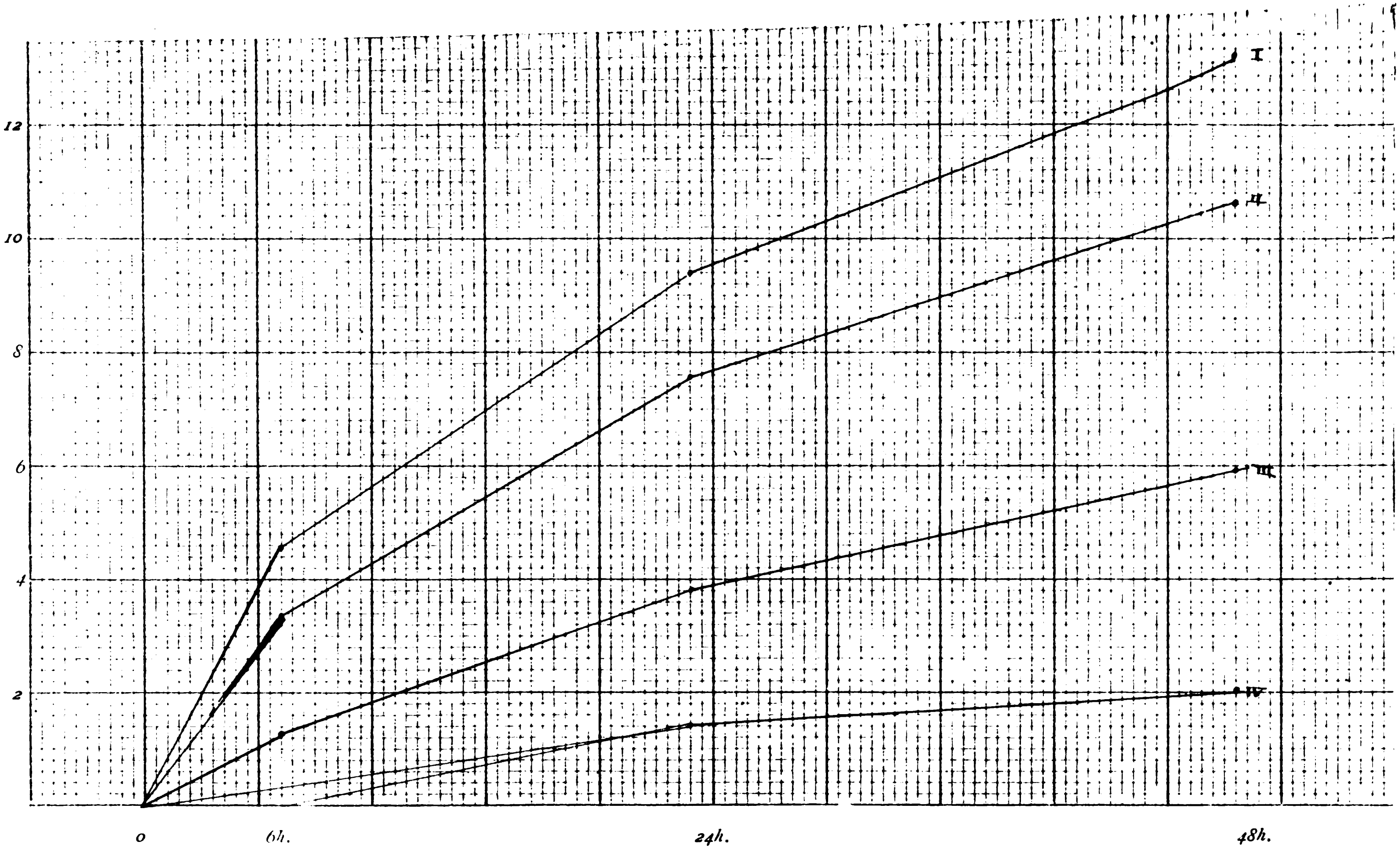


Saponin.

1/100 N. Solution { I From saline into 10% gelatin.
II From saline into 25% gelatin.

1/1000 N. Solution { III From saline into 10% gelatin.
IV From saline into 25% gelatin.

1/10000 N. Solution { V From saline into 10% gelatin.
VI From saline into 25% gelatin.



Solanin.

1/100 N. Solution { I From saline into 10% gelatin.
{ II From saline into 25% gelatin.

1/1000 N. Solution { III From saline into 10% gelatin.
{ IV From saline into 25% gelatin.

TABLE V.

| Period of Contact | Degree of Hæmolysis | $\frac{1}{100}$ N. Solanin |  | $\frac{1}{1000}$ N. Solanin |  |
|---|---|---|---|---|---|
|  |  | 25% gelatin | 10% gelatin | 25% gelatin | 10% gelatin |
| 1 hour | 30% | 0.14* | 0.09 | 1.5 | 1. |
|  | 5% | 0.125 | 0.085 | 1. | 0.65 |
| 2 hours | 30% | 0.09 | 0.075 | 1. | 0.8 |
|  | 5% | 0.085 | 0.07 | 0.7 | 0.55 |
| 4 hours | 30% | 0.07 | 0.055 | 0.7 | 0.6 |
|  | 5% | 0.05 | 0.04 | 0.5 | 0.4 |
| 24 hours | 30% | 0.03 | 0.025 | 0.03 | 0.25 |
|  | 5% | 0.025 | 0.02 | 0.25 | 0.2 |

* These figures indicate the quantity of supernatant saline required to produce the degree of hæmolysis stated.

The differences noted are not entirely easy of explanation; and it is especially difficult to account for the behavior of solanin, in that while diffusing much more readily than saponin, it should, at the same time, be so greatly influenced by concentration. These considerations apply only to the special case in which the hæmolysins are contained in the saline; when they are enclosed in the colloid and allowed to diffuse into saline the amount of diffusion is directly proportional to the concentration of the hæmolysins.

## DIFFUSION OF COBRA VENOM AND TETANUS TOXIN INTO COLLOIDS.

It has been stated that the hæmolysins of cobra venom and tetanus toxin pass from saline solution into gelatin and agar. A series of experiments was made to determine the effect of enclosing the toxins in one colloid (agar) and the blood in another (gelatin), and the reverse. Using a 5 per cent. suspension of dog blood in 10 per cent. gelatin and 0.1 per cent. cobra venom in 2 per cent. agar, it was found that complete hæmolysis occurred in twenty-four hours, while when the blood was mixed with the agar and the venom with the gelatin, the hæmolysis proceeded much more slowly. It was also ascertained that the venom hæmolysin passes more quickly into gelatin from agar than from saline solution. In this respect it agrees with the more definite chemical hæmolysins.

The next question considered was whether the entire series of venom principles pass into the colloids, and if so whether they diffuse with equal velocity. To test this the hæmolysed portion of the gelatin- and agar-blood mixtures was carefully separated from the other in which the blood corpuscles were still intact, and each portion was injected into guinea-pigs to determine the toxicity. In the case of the gelatin-blood mixture the portion to be injected intraperitoneally was first melted, and in the case of agar it was emulsified in saline solution. The gelatin used was 10 per cent., and the agar 2 per cent. for enclosing the venom, and 0.5 per cent. for enclosing the blood.

Two cubic centimeters of the venom-colloid mixture were used in each experiment. The number of minimal lethal doses contained in this quantity, for guinea-pigs, of about 180 grams weight, was sixteen. It was ascertained that three m.l.d. of the venom passed in the hæmolysed portion from the agar into the gelatin blood, and one m.l.d. from the gelatin into the agar blood. It was further ascertained that the non-hæmolysed portion of the colloid-blood mixture does not contain the cobra neurotoxin in appreciable quantities. In other words, cobra lysin and cobra neurotoxin diffuse together.

Since the two principles diffuse together it was desirable to ascertain, if possible, whether they diffuse accurately in the proportion in which they exist in venom; that is, whether the whole venom passes unchanged into the colloid, or whether a separation of the components occurs. It has been stated that, using two cubic centimeters of a 0.1 per cent. cobra venom suspension in agar, one fifth of its toxic constituents passed into the gelatin-blood mixture in twenty-four hours. It was ascertained that two cubic centimeters of the venom mixture contained 600 complete hæmolytic doses of cobra lysin (for dog blood). Since the rate of diffusion of cobra lysin is very little affected by time, as it proceeds almost in a straight line, all that is required to determine the quantity of diffused lysin is to multiply the amount of diffusion by the time. It was found by experiment that an amount of cobra lysin necessary to hæmolyse two cubic centimeters of a 5 per cent. suspension of dog corpuscles will require from three to four hours to pass from 10 per cent. gelatin containing 0.1 per cent. venom to saline solution; and that in twenty-four hours about twenty-four complete hæmolytic doses will have diffused into the saline. Hence the rate of diffusion of cobra lysin is as 24: 600 or 1:25. In the same time, as we have seen, the quantity of cobra neurotoxin which diffused from agar into gelatin was as 1:5. In other words, cobra neurotoxin diffused five times faster than cobra lysin from agar into a gelatin-blood mixture. This fact interesting in itself is rendered more suggestive in view of Faust's[11] recent statement of a relationship between cobra neurotoxin and the saponin substances.

A series of experiments similar to the above was made with a tetanus toxin one cubic centimeter of which contained 1000 minimal lethal doses for guinea-pigs of 250 grams and rats of 60 grams weight, and 33 minimal hæmolytic doses. It was found that both tetanolysin and tetanospasmin pass together easily into gelatin and agar, since the non-hæmolysed portions of the blood-colloid cylinder do not contain an appreciable quantity (less than 0.0001 c.c.) of the toxin. It would, however, appear from our experiments that tetanolysin diffuses more rapidly into colloids than tetanospasmin, since the great disproportion in quantity of spasmin and lysin—1000:33—which exists in the tetanus toxin does not bring about a corresponding preponderance of the spasmin in the colloid.

## SUMMARY.

Acids, alkalies, salts, glucosides, and certain toxins diffuse more quickly into 0.9 per cent. salt solution than into agar-agar and gelatin suspensions.

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[11] Die tierische Gifte, Braunschweig, 1906, p. 54.

The inhibitory effect of the colloids grows with increase in concentration, which increase affects both the velocity and extent of the diffusion.

In the case of gelatin the degree of diffusion is approximately in inverse proportion to the square root of the concentration. Agar-agar in strengths up to 2 per cent. inhibits far less than gelatin in 10 per cent. suspensions; and the difference in degree of inhibition exercised by 0.5 per cent. and 2 per cent. agar-agar is a small one.

Hæmolytic substances diffuse from gelatin into agar-agar more slowly than from saline into agar-agar. But the velocity of diffusion from agar-agar into gelatin is greater than from saline into gelatin.

The effects of differences in concentration of the hæmolytic agent vary according to the agent and the manner of its solution. When the hæmolyser is dissolved in salt solution the diffusion of $\frac{1}{100}$ N. and $\frac{1}{1000}$ N. solutions (saponin) is almost identical; while with solanin the stronger solutions diffuse faster. When the hæmolyser is dissolved in the colloid diffusion into fluid media is nearly proportional to the concentrations of the hæmolytic agent.

The velocity of diffusion into and from colloids is in general proportional to the square root of the time. Acids, alkalies, salts, and glucosides act in a manner which is in agreement with this rule. Cobra lysin and tetanolysin do not act in conformity with the rule.

Cobra lysin appears to diffuse into colloids more slowly, proportionally, than cobra neurotoxin, and tetanospasmin more slowly than tetanolysin.

The biological method described in this paper for studying diffusion in colloids is applicable to hæmolytic and some other toxic substances, and, with accuracy possibly only to such substances possessing relatively simple compositions.

Since all diffusion in the living body takes place within colloidal media of different concentrations, it would seem desirable to perfect methods through which the interaction of toxic chemicals and the fluids and cells of the body may in a manner be imitated *in vitro*. Through this means our knowledge of toxicology may well be extended.

# CLINICAL AND EXPERIMENTAL OBSERVATIONS UPON CHEYNE-STOKES RESPIRATION.

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## INTRODUCTION.

PLATES XXXI-XL.

In the course of a series of experiments upon cerebral compression, it was observed that respiration of the periodic type was not infrequent. Such periodic respiration may consist of smaller or larger groups of equal respirations, of respirations of gradually decreasing or gradually increasing magnitude, or finally of respirations of the Cheyne-Stokes type in which each group presents an ascending followed by a descending phase. The experimental and clinical basis of the following paper consists in a number of experiments in which periodic respiration was produced by cerebral compression, experiments with brain anæmia consequent upon ligation of the cerebral arteries, two clinical cases of Cheyne-Stokes respiration occurring in connection with increased intracranial tension, and eight cases of Cheyne-Stokes respiration occurring in other conditions. Only the results of clinical observation of the series of cases of Cheyne-Stokes respiration occurring in conditions other than that of increased intracranial tension will be given in this paper. The author is engaged upon a special investigation of such cases, and the results of experimental work already performed and future work will be reported subsequently.

## HISTORICAL.

It is not the purpose in this paper to give a general review of the literature of increased intracranial tension, except in so far as it bears directly upon periodic respiration in this condition. The extensive literature of Cheyne-Stokes respiration will be reviewed in a later paper.

There have been two distinct methods employed for the purpose of increasing the tension upon the brain substance. The first of these is known as "local compression," and consists in introducing a foreign body within the closed skull, by means of which a certain portion of the brain undergoes compression. The most convenient apparatus for this method, and the one that has been most frequently employed, is an elastic bag of thin rubber membrane which is connected with the end of a brass canula and introduced into a trephine opening in the skull, or through the lamina of one of the vertebræ. It is then filled with mercury under pressure, and any desired compression may be obtained and the amount be more or less accurately estimated. This method was first employed by Francois-Frank, and afterwards adopted by Horseley and Spencer, Hill, Cushing, and others. In the hands of all observers, such local compression has been followed by a variety of effects depending upon the locality of the brain upon which the compression was made. It has been shown by Hill,[1] Horseley and Spencer,[2] and others, that the brain does not transmit local pressure equally in all directions, and the effects observed have depended in a large degree upon the proximity of such compression to the medullary centres, or upon the position of the compression in so far as it may indirectly cause compression of these centres, as for example by forcing the medulla into the foramen magnum. This difference in effect is observed also, as Cushing[3] has pointed out, in local pathological processes observed clinically.

The other method for the experimental production of cerebral compression is known as "general compression." It consists in running into the closed cranio-vertebral canal fluid, consisting of defibrinated blood, Ringer's solution, water, or an isotonic solution of sodium chloride. This method has been variously employed; some observers have injected the fluid over the cortex and others through the lower end of the spinal canal while others again after trephining the lamina of one of the vertebræ have introduced the fluid directly over the medulla or some portion of the spinal cord. Naunyn and Schrieber,[4] in their extensive experiments upon this form of compression, distinguished two methods, in one the compressing fluid being allowed to enter the space between the skull and dura ("extrapial compression"), or in the other, the subarachnoid space ("intrapial compression"). They observed no difference in results by these two methods.

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[1] The Physiology and Pathology of the Cerebral Circulation, London, 1896. *Proc. Roy. Soc.*, 1894, lv, 52.

[2] *Trans. Royal Soc., London*, 1892.

[3] *Mitteilungen aus den Grenz. der Med. und Chir.*, 1902, ix, 791.

The earlier authors observed, as the result of an increase in intracranial tension, certain characteristic symptoms, such as slow pulse, spasms of the Kussmaul-Tenner type, and various respiratory changes.[5] By the majority of these authors it was stated that to produce death it was necessary to raise the tension to a point equal to carotid pressure, and the effects observed were referred in the greater number of instances to cerebral anæmia. Naunyn and Schrieber[6] in the course of a large number of experiments, with compression, in which the tension was increased by the general method, observed that in some cases there resulted from the increase in intracranial tension a rise of arterial blood pressure, which in a few experiments reached 280 mm. Hg. or even more; death resulted in all instances when the compression was continued. They observed that the blood pressure frequently took the form of waves. They noted that the respirations, if these were abolished upon raising the intracranial pressure, usually recurred at the crest of the subsequent blood pressure wave. Lowering the intracranial pressure at any time was followed by a fall in blood pressure.

Horseley and Spencer employed the local method of compression. They analyzed minutely the results of compression over different portions of the brain and cord. They observed the dependence of the respiration upon the blood pressure, and found that a rise of blood pressure often caused renewed respiratory activity after respiration had ceased for some time.

Hill produced compression by both the local and general methods. He agrees with other observers in the conclusion that when the pressure is gradually raised, fatal symptoms do not occur until the pressure of the introduced fluid is almost equal to the arterial blood pressure. He concludes that the symptoms which are observed are entirely due to cerebral anæmia, and that the pressure *per se* has no effect in exciting the bulbar centres. He divides these symptoms into two kinds, excitatory and paralytic. When the vagi were cut or paralyzed, or when the cardio-inhibitory centre was "tired out" by several successive elevations of intracranial pressure, enormous increase of blood pressure was the first result. These striking "excitatory" symptoms he believed are never caused by pathological processes occurring clinically, because compression is too low at the onset. In the "paralytic" stage, Traube-Hering waves of blood pressure and Cheyne-Stokes respirations were frequent, especially in the period of recovery after the compressing agent had been removed. He observed that respirations accompanied the rising limb of the waves of blood pressure and the apnœic periods were associated with the fall of blood pressure. In one clinical case, the nature of which he does not state, he showed by means of the plethysmograph that there was dilatation of the vessesl of the arm during the apnœas, and constriction during the dyspnœas.

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[4] *Arch. f. exper. Path. u. Phar.*, 1882, xiv, 1.

[5] For a resumé of the older history of cerebral compression, see Hill, *loc. cit.*

[6] *Loc. cit.*

Cushing,[7] in a series of experiments in which the amount of intracranial pressure was recorded coincidently with the blood pressure, was the first to clearly associate the blood pressure changes with the degree of intracranial tension. He was able to show in a very striking way that the arterial blood pressure is related to the intracranial pressure as a compensatory process. When intracranial pressure exceeded the blood pressure, a rise of the latter occurred, and this tended to remain slightly but constantly above the line of intracranial tension; the result of such a rise of blood pressure was obviously a protection against bulbar anæmia. So long as this relation was maintained, disturbances of pulse and of respiration and cerebral symptoms were in great part absent, and death was not imminent until the blood pressure was no longer held above the pressure of the compressing fluid. He showed that this rise of blood pressure did not occur after section of the spinal cord above the origin of the splanchnics, nor after cocainization of the medulla, and by direct observation of the splanchnic vessels, he was able to see that with the rise in blood pressure these vessels underwent constriction. Employing a cerebral window placed in a trephine hole in the skull, he observed that the circulation of the blood in the capillaries of the cortex varied in accordance with the relations existing between the blood pressure and the intracranial pressure. When intracranial pressure was greater than the blood pressure, the capillaries were blanched, and the rise of blood pressure above the intracranial pressure line was coincident with a renewal of the blood supply to these vessels. He observed the frequent occurrence of Traube-Hering waves during such experiments, and found that the respirations accompanied the rising limb of these waves and disappeared with falling blood pressure; in this way respiration of the periodic type may occur. By a number of clinical observations[8] he demonstrated the blood pressure reaction in states of increased intracranial pressure, and in Cheyne-Stokes respiration occurring in two of these cases, was able to determine by means of the Riva-Rocci sphygmomanometer that the occurrence of respiratory activity was coincident with a rise of blood pressure, the apnœic periods with a fall.

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[7] See also "The Mutter Lecture," *American Journal of the Medical Sciences*, 1902, cxxiv, 375, and *Johns Hopkins Hosp. Bull*, 1901, xii, 290.

## METHODS.

In order to produce cerebral compression experimentally I have employed, except in a few cases, the general method, and have recorded the intracranial pressure coincidently with the blood pressure by the method suggested by Cushing. A trephine opening was made over some portion of the skull or through the axis, the dura carefully excised to the full extent of the opening, and into the opening in the bone was screwed an accurately fitting brass canula which cut its own thread as it entered. The canula was connected by means of lead tubing of small bore with a T-piece. One arm of the T passed to a mercury manometer, the other arm was connected with a pressure bulb by means of which the pressure in the system could be increased, and the increase measured by the manometer. Between the brass canula and the T, a part of the lead tubing was coiled and lay in a beaker of water which was warmed to body temperature. A mercury pressure apparatus was interculated in the system for producing very high pressure. The apparatus is shown in the diagram. After the screw clamp "a" is closed, the pressure in the system may be increased by raising the pressure bulb. By closing the screw clamp "b" and opening "a," mercury pressure may be employed.

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[8] The Blood Pressure Reaction of Acute Cerebral Compression, illustrated by Cases of Intracranial Hæmorrhage, *American Jour. of the Med. Sciences*, 1903, cxxxv, 1017.

The blood pressure in the carotid or femoral was recorded by means of another manometer, the pen of which wrote as nearly as possible in the vertical line of that recording intracranial pressure. The zero pressure of each system was first determined, and the manometers so arranged that the zero of each was represented by the same base line. Hence when the two manometers were recording at the same height, each system was under the same pressure. It is only by this means that an accurate comparison can be obtained between the changes of intracranial pressure and of blood pressure. The animals employed were dogs. Observation of the cortex was made directly by means of an accurately fitting glass window, screwed into a trephine opening in the skull over some portion of the cortex under which the dura had been excised. It is important that the dura under the trephine opening through which the compressing fluid enters should be carefully excised, in order to insure that the fluid enters the subarachnoid space and thus has free access to all parts of the brain. If only a slit be made, the first result of the compression may be to close this opening like a valve; the fluid is then prevented from entering and is confined to the area between the skull and the dura.

In the cases of Cheyne-Stokes respiration occurring in man, continuous records of blood pressure and pulse rate showing their relation to the respiratory changes were obtained. The blood pressure records were obtained by means of the sphygmomanometer

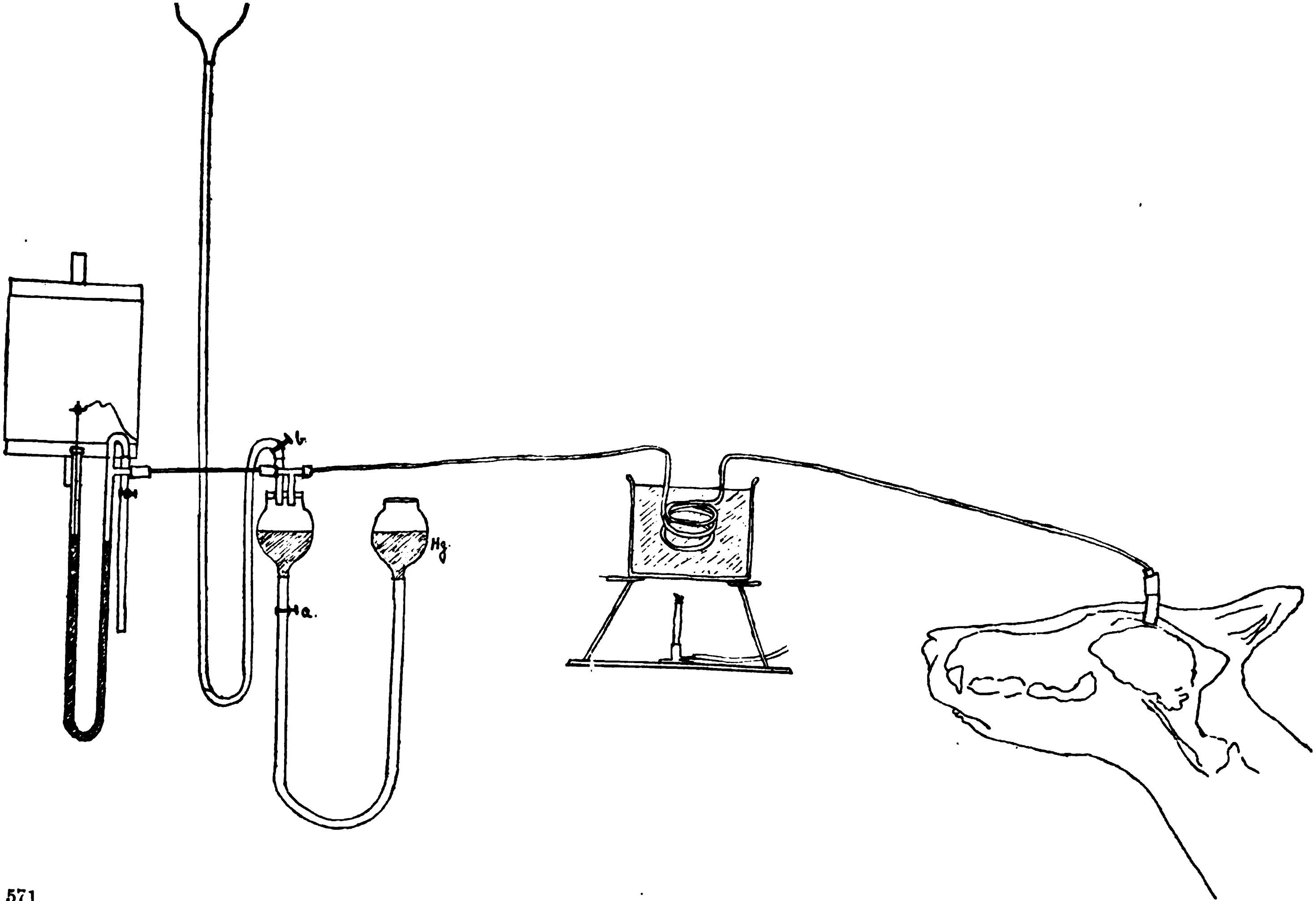

FIG. I.

devised by Erlanger,[9] and the method employed was as follows: The systolic and diastolic pressures in the brachial artery were first accurately obtained and an average of the two made. The instrument was now set at this point and a continuous record of the pulse taken. With this instrument the size of the pulsations of the recording pen is greatest when the pressure exerted by the instrument is equal to the diastolic pressure, and progressively decreases as the pressure in the cuff is increased above this point. Now if the instrument is set so that the pressure exerted upon the arm is half way between systolic and diastolic pressure, a rise or a fall of the blood pressure will affect the size of the pulsations. A rise of blood pressure, by bringing the diastolic pressure nearer the point at which the instrument is set, increases the size of the pulsations, while a fall of pressure by making the diastolic pressure more distant from this point, has the opposite effect. If the blood pressure remains constant, a slowing of the pulse rate also tends to increase the size of the pulsations. The slowing of pulse rate that occurred in these cases was always associated with a fall of blood pressure, so that the fall of pressure shown by the pulse record was probably greater than the instrument indicated. So also a more rapid pulse was associated with the elevations of blood pressure, and hence such rises were probably greater than they appear on the records as the result of the mere increase in size of the pulsations.

So far as I know, this, with one exception, is the first time that a continuous record of blood pressure has been made during Cheyne-Stokes respiration in man. Mosso,[10] in a recent publication, gives tracings of such records obtained during periodic respiration occurring in normal men at high altitudes. Indeed, the Mosso and Erlanger instruments are the only two sphygmomanometers that permit of such use, and for this purpose the latter is certainly better adapted than the instrument devised by Mosso. The importance of such records in clinical cases of many kinds is too evident to need emphasis, and the method opens up a new field for much valuable investigation. In the clinical cases which I have studied, quantitative determinations of the blood pressure at its maximum and minimum variations were made by the Riva-Rocci method in some cases. The respirations were recorded by means of Marey's double tambour, the upstroke representing inspiration.

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[9] *Johns Hopkins Hosp. Reports*, 1904, xii, 53.

[10] *Arch. ital. de biol.*, 1905, xliii, Fig. 21.

*Discussion of the "General Method" of Cerebral Compression.*—Two principal objections have been made to this method. In the first place, it has been contended that the compression is not exerted equally upon all parts of the brain, or it has even been claimed that certain portions may entirely escape compression. Thus it is stated that if the fluid is allowed to enter over the cortex, the cerebellum may be crowded down into the interior opening of the foramen magnum to such an extent that the medulla is protected in large part or entirely from the effects of compression. Hill especially has offered this objection to the employment of the general method. By opening the occipito-atlantal ligament and introducing fluid under pressure over the parietal region, he observed that in many cases none of the fluid leaked through the foramen magnum. He believes that the great brain is driven downward against the base and completely blocks the isthmus tentorii cerebelli, and at the same time the cerebellum and medulla descend and block the foramen magnum. I believe, however, that this experiment cannot be regarded as crucial, for when one thus opens any part of the cranio-vertebral canal and allows the escape of the cerebro-spinal fluid, the brain and cord no longer fill with entire completeness their respective cavities. This is especially true if the opening is made over an underlying portion, such as the medulla, for one thus drains the whole cranial cavity. The occipito-atlantal ligament being opened and the fluid drained off, pressure exerted above may force the brain downward as Hill observed, but under normal conditions this displacement is in large part prevented by the practically incompressible fluid in the spinal canal, which has no way of escape except to a small extent along the sheaths of the spinal nerves. Hill states that the normal amount of cerebro-spinal fluid is extremely small. In all the animals that I have observed, however, it has been present in considerable quantities, and flows out readily upon opening the dura.

If one trephines a hole through the axis and another through the parietal region of the skull, and introduces the compressing fluid through the former, the brain will be forced up and become closely applied to the opening in the parietal region, and with an increase in pressure will bulge through it and effectually prevent any escape of fluid. The same result may be observed if the two openings are made close to each other over the cortex. If an attempt were made to measure by ordinary means the pressure transmitted to the second trephine opening, as for example with a mercury manometer, the opening would be soon blocked off, owing to the crowding of the brain towards this point of lessened pressure. To prevent this result as far as possible, I have employed the following method. The compressing fluid is introduced through one trephine opening and the second opening is connected by means of lead tubing of small bore with a Hürthle spring manometer. The tube connected with the Hürthle manometer is completely filled up to its opening into the skull or spinal canal with fluid under slight pressure. The pressure of the entering fluid is measured with a mercury manometer by the usual method. The pressure transmitted to the distant portion of the cranial cavity is measured by the Hürthle manometer. The tube connecting with the latter being filled with a practically incompressible fluid, and the changes in pressure that it records being associated with comparatively slight movements of this fluid, a point of lessened pressure is in large part avoided, and the brain is thus prevented to a considerable degree from being forced into and plugging the trephine hole.

By the employment of the above method, I was able to observe that the pressure over the medulla, when the fluid entered over the cortex, was equal in degree to that over the cortex, and was approximately coincident. The pressure over the medulla followed in a few seconds the pressure of the entering fluid, and a slight pressure (about 20 mm..), remained for a short time over the medulla after removal of the compression over the cortex. When the brain had been submitted to pressure for some time, the agreement between the pressures was not so close, and the pressure that remained over the medulla after the pressure bulb was lowered was increased.

When the fluid was allowed to enter over the medulla and the measurement was made over the cortex, the correspondence was just as close until a certain point was reached, when the Hürthle manometer suddenly ceased recording, and remained stationary though the pressure of the entering fluid continued to increase. This phenomenon was evidently due to a sudden blocking of the trephine opening by the brain.

Investigation has shown the anatomical and physiological continuity of the subarachnoid space of the skull and spinal canal with the cavities within the brain itself. The fact that there is little difference in the effect observed when the primary entrance is over the medulla and when it is over the cortex, would indicate in itself that the pressure is well transmitted. Cushing, by the employment of both methods in a large number of cases, observed that the results were practically identical, and were due in both cases to the effects of medullary compression. I can confirm this opinion by the result of a large number of experiments, in which fluid was allowed to enter in some cases over the cortex, in others over the medulla. The only exceptions occurred toward the end of long experiments. When the intracranial pressure has been kept high for a long period of time, the brain may become markedly œdematous. It is probable under these circumstances that more or less blocking may occur at the isthmus tentorii cerebelli, or at the cranio-vertebral junction; the pressure upon the medulla is then indirectly transmitted by crowding the hind brain down into the spinal canal, rather than exerted directly by the compressing fluid. This condition is expressed in the results of the experiments by a lack of the usual close correspondence between the effects observed and the relations of intracranial and blood pressures, as will be more evident after a consideration of the following sections.

The effects upon the respiratory, vasomotor and cardio-inhibitory centres, occur more rapidly when the fluid enters directly over the medulla than when it enters over the cortex, and the delay is readily explained by the fact that the transmission of pressure is not immediate in the latter case, as is stated above.

The second objection urged against the method of general compression, is that the escape of fluid through the free openings existing between the subarachnoid space and the sinuses, as well as along the sheaths of the spinal nerves, is so rapid that the degree of intracranial tension can not be kept constant, and moreover that the entrance of this great amount of fluid into the circulation, causes dilatation of the right heart and the death of the animal. This view has been especially advocated by Adamkiewicz[11] and Hill. The former states, largely upon theoretical grounds, that any tendency of the cerebrospinal fluid to assume a higher tension than normal, is overcome by the passage of a certain amount of this fluid into the blood. Cushing however, by the employment of this method in numerous cases, observed that as a matter of fact this objection is not a serious one. The escape of fluid during a given time was not great (60 to 100 cc. in the course of an hour), and certainly not sufficient to afford ground for either of the difficulties suggested by Adamkiewicz. The escape of fluid after death of the animal was somewhat more rapid. Both of these statements I can confirm as a result of my experiments.

## PERIODIC RESPIRATION EXPERIMENTALLY PRODUCED BY INCREASE OF INTRACRANIAL TENSION. DESCRIPTION OF TRACINGS.

When the intracranial pressure is gradually increased from zero, there is as a rule no effect upon respiration until the pressure of the compressing fluid rises almost to the mean blood pressure as measured in the carotid or femoral. In some cases there is a slight increase in respiratory rate and depth before this point is reached, especially if the intracranial pressure is held for some length of time at a point somewhat below arterial pressure. When the intracranial pressure reaches mean arterial pressure, there is observed in a few seconds (one to five) a preliminary rapid increase in respiratory rate and depth. This increase, lasting a few seconds, is followed by a rapid diminution in the rate and depth of respirations, leading to apnœa in from two to ten seconds. The preliminary increase may be absent. Accompanying the decreasing respirations and the apnœa, there is usually a development of vagus inhibition associated with a fall in blood pressure which varies greatly in extent in different cases. This fall is followed in turn by a rise of blood pressure and the partial disappearance of vagus inhibition, though the latter persists in nearly all cases to a greater or less degree, the pulse being nearly always somewhat slower throughout the compression than it was previously. The blood pressure then gradually rises toward the line of intracranial pressure, and after rising above this line at the summit of the rise, the apnœa is broken. If the blood pressure does not again fall, but remains constantly above the line of intracranial tension, respirations continue, usually somewhat slower and deeper than normally, but with entire regularity.

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[11] *Neurolog. Centralbl.*, 1897, xvi, 434.

Periodic respiration is observed when the blood pressure alternately rises and falls above and below the line of intracranial tension in the form of Traube-Hering waves; the periods of respiratory activity accompanying the rise, the periods of apnœa the fall. This condition may as a rule be obtained in any given experiment by successively raising the intracranial pressure through several stages, each time to the height of mean blood pressure. Following the first increase of intracranial tension to the level of arterial pressure, the blood pressure rises, assumes a height somewhat greater than the former, and usually remains constantly above the intracranial pressure. If the intracranial pressure is now again raised to the arterial pressure at this new height, the same process may be repeated. A third successive increase of the intracranial pressure to blood pressure may lead to a similar result, or the blood pressure rise which follows may not continue, but may be succeeded by a fall to below the line of intracranial tension. This fall in turn is followed by a rise, and the condition of blood pressure waves rising and falling above and below the line of intracranial pressure is obtained.

Less frequently this condition may follow the first or second increase of intracranial tension (Plate XXXI, Tracing 1). These blood pressure waves represent periodic variations in the activity of the vasoconstrictor centre. To maintain this unusually high blood pressure, a very strong stimulus to the centre is necessary. As will be shown later, the fall of blood pressure below the intracranial pressure is associated with anæmia of the medullary centres. This acts as a strong stimulus to the vasoconstrictor centre, the centre responds, and a rise of blood pressure results. With the rise of blood pressure above the line of intracranial pressure, the anæmia is greatly reduced, and the stimulus acting upon the centre is correspondingly lessened. The action of the centre therefore partially relaxes and a fall of blood pressure occurs. With the fall below the line of intracranial pressure, the stimulus is again increased and the same process is repeated.

This condition of blood pressure waves rising and falling above and below the line of intracranial tension, is always associated with periodic respiration. The first blood pressure wave following the increase of intracranial tension may in some cases fail to call forth respirations. This phenomenon is observed when as a result of pronounced vagus inhibition following the rise of intracranial tension, the blood pressure fall has been great and long continued. Such fall of pressure leads to a long continued anæmia of the respiratory centre, and as a result its irritability may be so depressed, that renewed activity may not be observed until after a considerable interval of blood supply; in this interval may be included the first blood pressure wave. This fact will be more evident I think after consideration of further points to be dealt with in this paper.

The number of respirations in a group varies greatly; their character and sequence in different experiments also varies, though in the same experiment there is usually considerable constancy. Two to ten or more respirations may be present in a group; rarely some of the waves of blood pressure may be productive of only a single respiration, preceded and followed by groups of two or more. The groups may be composed of respirations of approximately equal size; more frequently they present an ascending scale; still more frequently the first respiration is the largest and the group presents a descending scale. Not infrequently the groups may assume a more or less typical Cheyne-Stokes character, with an ascending and descending phase in each group.

*Tracing 1* shows groups of two respirations separated by long periods of apnœa, the first respiration of each group being the larger. The uppermost line of the tracing records the abdominal respiratory movements.[12] One of the two middle lines represents the blood pressure in the carotid artery, the other the intracranial pressure, the latter being the more continuous line. Both pressures are recorded from the same base line (not shown on the record). The speed of the kymographion was 0.5 m.m. per sec. The record reads from left to right, the pen recording respiration records 5 mm. ahead of the pen of the manometer recording blood pressure, and its downstroke represents inspiration. Following the increase of intracranial pressure, which was gradually raised from zero, a slight preliminary increase in respiratory rate was observed; soon there was a decreasing scale ending in apnœa and associated with the development of vagus inhibition, and a fall of blood pressure. A slow rise of blood pressure now followed, and apnœa was broken by a single respiration about twenty seconds after the crest of the blood pressure wave rose above the line of intracranial tension. This was succedeed by a fall of blood pressure and apnœa, and a following rise of blood pressure was attended by a group of three respirations. The intracranial pressure was now still further raised, and a long period of apnœa occurred, during which the blood pressure gradually rose. This period of apnœa was finally terminated by a group of two respirations. These were succeeded in turn by a fall of blood pressure and apnœa; another group of respirations occurred with the succeeding rise, and periodic respiration with groups of two respirations separated by long periods of apnœas was then definitely established (see Tracing 1). The blood pressure slowly rises throughout each period of apnœa, and at the summit of the rise the first respiration of the following group occurs. The occurrence of the first respiration is marked by a further almost immediate rise of blood pressure and an increase in the pulse rate. Following the first respiration there is an equally sudden fall of pressure. The second respiration of the group now occurs, attended by a similar rise of blood pressure and increase in pulse rate, and followed by a fall of pressure to below the line of intracranial tension. Apnœa now begins, during which there occurs a gradual rise of blood pressure and a progressive slowing of the pulse, continuing up to the first respiration of the succeeding group. The points to be especially noted in this experiment are the following: (*a*) The condition present is one of long periods of apnœa and few respirations in a group, and the rise of blood pressure precedes for a long time the beginning of respiratory activity. (*b*) There is partial removal of vagus inhibition during the periods of respiration and its gradual development during apnœa. An average of four counts of the pulse rate gave eighty-nine for the former and eighty-one for the latter. (*c*) As the intracranial pressure was allowed to fall slowly and gradually throughout the experiment, the depth to which the blood pressure fell below the line of intracranial pressure after each respiratory group successively decreased, and this change was associated with a decrease in the length of the periods of apnœa; finally a group of four respirations occurred, following the shortest period of apnœa.

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[12] The thoracic respirations were also recorded and were identical.

*Tracing 2* shows groups composed of from two to six respirations separated by much shorter periods of apnœa. The blood pressure shows relations similar to that observed in the last experiment, except that it does not fall so far below the line of intracranial tension. Each respiration is accompanied by a rise of blood pressure and removal of vagus inhibition, and is succeeded by a fall of blood pressure, the fall following the last respiration of the group being the most marked.

*Tracing 3* shows groups of from four to eleven respirations, usually of increasing magnitude. Several of the groups present also a subsequent descending scale of respirations and approach more closely the true Cheyne-Stokes character. Following the rise of intracranial pressure from zero there was a rise of blood pressure associated with increased respiratory activity. The blood pressure fell only slightly following this rise, and no apnœa occurred, but only a slowing of the respirations. These slow respiratory movements continued for a time, and the last respiration was followed by a sudden fall of blood pressure to far below the line of intracranial pressure. With this fall apnœa developed and lasted for thirty-four seconds, during which time the blood pressure gradually rose; following this rise a group of respirations occurred. Following this group, the blood pressure fell slightly, and a slowing of the respirations occurred. A third group now occurred, and periodic respiration with definite intervals of apnœa developed. The following results of this experiment are to be emphasized: (*a*) Certain groups of respirations are preceded by a slight rise of blood pressure; in other groups the beginning of respiratory activity and the rise of pressure are practically coincident; in others again the former precedes slightly the beginning of the rise of blood pressure. (*b*) With the longer periods of apnœa, there is usually a rise of blood pressure observed before the following group of respirations, while with the shorter periods of apnœa, the beginning of respiratory activity is usually coincident with or slightly precedes the rise in pressure. (*c*) Each respiration of the group is accompanied by a rise of blood pressure and an increase in the pulse rate, and following each respiration there is a gradual fall of blood pressure and a decrease in pulse rate, and these changes gradually increase up to the beginning of the next respiration of the group. (*d*) The final respiration of the group is succeeded by a very rapid fall of blood pressure to far below the line of intracranial tension, and with this fall apnœa develops. (*e*) The longer periods of apnœa are as a rule associated with the most marked fall of blood pressure. In the shorter intervals of apnœa the blood pressure does not fall to so great an extent below the line of intracranial tension. (*f*) The largest respiration of the group is associated with the greatest rise in blood pressure. (*g*) The fall of blood pressure following each respiration of the group is associated with the development of vagus inhibition, and this inhibition reaches its greatest development with the final fall following the last respiration of the group.

Inspiration in this and in the last two tracings is represented by the downstroke of the respiratory pen. The records are to be read from left to right. In this record, the respiratory pen records two millimeters ahead of the pen of the blood pressure manometer.

*Tracing 4.*—The respiratory groups in this record are of the typical Cheyne-Stokes type with an ascending and descending phase to each group. This record was obtained from a dog after the section of one vagus. The lowermost line is the line of zero pressure for both the blood pressure and intracranial pressure. The darker of the two middle lines represents the intracranial pressure, the lighter, the blood pressure. The pen recording the former records a few millimeters behind the other pens. The record reads from right to left. The time is in one second intervals. The record is reduced one half. Inspiration is represented by the upstroke of the respiratory pen. The rise of intracranial pressure from zero was followed shortly by a rise of blood pressure, and accompanying this rise a group of five respirations occurred. A considerable fall of blood pressure associated with the development of vagus inhibition now followed. This fall of pressure was accompanied by apnœa lasting seventeen seconds, and was followed by a group of four respirations associated with a second rise of blood pressure and increase of pulse rate. The blood pressure then fell, but did not reach to a point so far below the intracranial pressure as previously, and the apnœa that accompanied this fall was of only six and a half seconds in duration. Associated with the rise of the third blood pressure wave, a third group of respirations occurred. The blood pressure following this last group fell somewhat, but not to so low a point as in the previous periods of apnœa. The result was that no apnœa followed, and its place was taken by a series of small respirations which increased in size gradually as the blood pressure rose. There then followed a slight fall of blood pressure with slight diminution of the respirations; finally, as blood pressure waves disappeared and the blood pressure remained constantly above the line of intracranial pressure, the respirations continued at regular intervals and shortly afterward became equal in depth. In this record it is also to be noted that, in the long period of apnœa preceding the second group of respirations, a rise of blood pressure occurred before the commencement of respiratory activity, while in the shorter periods of apnœa, the group of respirations preceded the rise.

*Tracing 5* is a record of periodic respiration in groups of twos and threes occurring after section of both vagi. Under these conditions, the pulse rate is the same in the periods of apnœa and in the periods of respiratory activity.

## REPORT OF RECORDS AND OBSERVATIONS UPON TWO CLINICAL CASES OF CHEYNE-STOKES RESPIRATION OCCURRING WITH INCREASED INTRACRANIAL PRESSURE.

*Case I.*—G.W., male, colored, aged 22 years, was admitted to the surgical service of the Johns Hopkins Hospital on Dec. 6, 1905, with a history of headache and recurring clonic spasms of the left side of the body of nine months duration. The left eye was the seat of a marked choked disc. The patient was first seen by me on the night of Dec. 8. At that time and on the following afternoon, his condition was as follows: There was marked periodic respiration with long periods of apnœa. The patient was restless, talked irrationally, complained of pain in head, and moved limbs and body during the respiratory periods, though his attention could be drawn and he would answer questions fairly intelligently. With the onset of apnœa, his eyes assumed a fixed stare, the muscular movements of the body and limbs ceased, and in a few seconds there was dilation of the pupils with rotation of the eyeballs outward and upward. Unconsciousness now occurred, from which it was impossible to arouse the patient. An average of three determinations of the pulse rate gave in the periods of respiratory activity 115 per minute, during the periods of apnœa 61 per minute. During the respiratory periods the patient did not seem to be especially dyspnœic, and did not complain of shortness of breath. On the morning of Dec. 10, the periods of apnœa were shorter and the periods of respiratory activity longer. At times respiration would continue without pauses for several minutes. The patient was very restless and his mental condition was more active than the day before, though he was still very irrational. Each apnœic period as previously was associated with total unconsciousness. On the afternoon of this day his apnœic periods had almost entirely disappeared, only three short ones of 10 to 20 sec. being noted in the course of an hour. The patient was still very restless, his mental condition even more active than at any previous time, and he was constantly muttering and holding imaginary conversations. No periods of unconsciousness occurred except those associated with the few and short periods of apnœa. On the morning of Dec. 11 the mental condition was much improved and the breathing was regular in force and rhythm. This condition continued until the following morning, when the patient was removed to the operating room and decompression performed. Generalized tuberculous meningitis with marked increase in intracranial tension was found. Shortly after the operation it was definitely noted that the choked disc had undergone partial resolution. The patient remained in a partially unconscious condition the following two days; there was delirium, and death occurred on the sixth day following the operation. Autopsy revealed a solitary tubercle of the cerebrum, tuberculous meningitis, and tuberculous pneumonia. After the operation the breathing remained quite regular, and no return of apnœic periods occurred.

A study of the numerous records taken at different times during the period of observation shows that the periods of respiratory activity may consist of groups of respirations of approximately equal depth, of descending groups in which the first inspiration is the largest, of groups of respirations of irregular depth, and finally of groups which present the typical ascending and descending character. The groups of irregular respirations and of those of the gradually decreasing type are probably the most common. The number of respirations in a group varies: there may be only two, or respiration may be continuous for many minutes, though as a rule, at any one time, the number of respirations in the groups is fairly uniform and the periods of apnœa are of approximately equal length. In the above case, the periods of apnœa varied at different times from a few seconds to a minute or longer. Tracing 6 (Plate XXXIV) shows a group of two respirations, the first of which is the larger. A typical descending group of three, which was often observed, is shown in Tracing 7. Tracing 8 shows a group of irregular respirations, a group of three descending respirations, and a group in which the respirations are approximately equal. Tracing 9 shows groups of a typical Cheyne-Stokes character, with an ascending and descending phase. Tracings 10 and 11 are examples of descending groups.

The upper line of the records is the blood pressure from the brachial artery, recorded by means of the Erlanger sphygmomanometer by the method above described. Any increase in the size of the pulse wave indicates a rise in pressure, any decrease a fall. The time is in one second intervals. The middle line is the respiratory record, the upstroke representing inspiration. It will be noted that the groups of respirations are constantly associated with a rise in blood pressure and a rapid increase in the rate of the pulse. In some cases the rise may precede the beginning of respiratory activity, in others it may quickly follow it, in others the two are coincident. A careful study of numerous tracings has led to the following general conclusions:

(1) In tracings taken at any given time, when conditions may be assumed to be fairly constant, the respiratory groups following long periods of apnœa are usually preceded by a rise in blood pressure, while with shorter periodsof apnœa the respirations precede slightly the rise. This is illustrated in Tracing 8. The first respiratory group followed an apnœa of twenty seconds and the rise of blood pressure was practically coincident with the beginning of respiratory activity. The second group of three respirations followed an apnœa of six seconds, and preceded the rise of blood pressure by about two seconds. The succeeding apnœa of thirty-four seconds was preceded by a rise of blood pressure of about three seconds.

(2) The rise of blood pressure is proportional to the depth and number of respirations in a group: the greater and more long continued the rise, the greater the respiratory activity.

(3) The longest periods of apnœa are associated with the

greatest falls of pressure, and the most marked slowing of the pulse rate.

(4) The most marked increase in pulse rate occurs when the rise of blood pressure is most marked.

(5) As a rule, the descending groups in which the first respiration is the largest, are preceded by a marked and sudden rise of blood pressure and by a sudden increase in pulse rate, while the rise preceding or accompanying the other groups is usually more gradual, and the increase in pulse rate is not so marked before the first respiration occurs. Tracings 7 and 11 illustrate this point.

(6) If the rise of blood pressure precedes the respiratory group, this rise is associated with an increase in pulse rate; but if such a rise does not occur previous to respiratory activity, the pulse is not increased in rate until the beginning of respiration.

(7) Following the respiratory groups there is a fall of blood pressure, and a gradual slowing of the pulse which reaches its fullest development coincident with the lowest point of blood pressure.

(8) With the disappearance of the periodicity of the respiratory activity, there is a disappearance of the blood pressure waves and of the variations in pulse rate. Tracing 12 is a record of the blood pressure and pulse rate taken from the patient at a time (afternoon of Dec. 10) when the respirations were entirely regular in force and rhythm. The small respiratory waves are seen, a slight rise with each inspiration and a slight fall with expiration, but otherwise the blood pressure and pulse rate remain constant, and there is an entire absence of the long blood pressure waves noted during the time of periodic respiratory activity.

The points to which I wish to call especial attention in this case, exclusive of those shown by the tracings, are: (1) The dilatation of the pupils and the rotation upward and outward of the eyeballs with the onset of apnœa. These phenomena were noted by Cushing[13] in the cases reported by him. (2) The deep unconsciousness during the periods of apnœa, and the mental and physical activity during the periods of respiration. (3) The blood pressure variations during the course of observation.

[13] *Loc. cit.*

The periods of consciousness and activity were associated with the rises of the blood pressure waves, the periods of unconsciousness with their fall. If we regard the waves of blood pressure as rising above the intracranial pressure during the former and falling below this line during the latter, in accordance with the view which the experimental work makes very probable, the deep unconsciousness is associated with a lack of blood supply to the brain, a condition of cerebral anæmia, and the following period of mental and physical as well as respiratory activity, with a renewed supply of blood to the cerebral arteries. This point will be more clearly demonstrated by the following section

The blood pressure during the different days of observation is given in the following table. It was obtained in all cases during respiratory activity.

| | | | Systolic. | Diastolic. |
|---|---|---|---|---|
| December | 8. | 9.30 P.M. | 122 | 98 |
| " | 9. | 4.30 P. M. | 130 | 100 |
| " | 10. | 10.00 A. M. | 138 | 110 |
| " | 10. | 3.30 P. M. | 150 | 110 |
| " | 11. | — — | 150 | 120 |
| " | 12. | Before opeation | 162 | 132 |
| | | During operation | 170 | 136 to 114 |
| | | 1½ hours later | 135 | 90 |
| | | 4 hours later | 138 | 96 |
| | | 6 hours later | 140 | 98 |

It will be noted that a rise in blood pressure was observed with the gradual improvement of the patient's respiratory, mental, and physical condition. On Dec. 8, the periods of unconsciousness were long, in some cases reaching a minute or even more, and the periods of respiratory activity were short. The condition was slightly improved the following day, the length of the respiratory periods being somewhat increased at the expense of the periods of apnœa. On the morning of Dec. 10, there was a considerable improvement noted: the apnœic periods were shorter and over a given length of time fewer in number, the respiratory periods were longer and the mental activity greater. These changes, as may be seen, were associated with a rise of both systolic and diastolic pressures. On the afternoon of this day, there was noted an almost complete absence of apnœic periods, the patient was very restless, and mental activity was almost constant and very marked.

There was an associated rise of systolic pressure of twelve millimetres over that observed in the morning. On the following two days absolute disappearance of the periods of apnœa and unconsciousness occurred. On the second of these days, there was a further rise of blood pressure. Following the decompression, there was an almost immediate fall of diastolic pressure, though the systolic pressure remained quite constant throughout the operation. One and a half hours later, a fall of systolic pressure of thirty-five millimetres of mercury was observed, associated with a still further fall of the diastolic pressure. This gradual rise of blood pressure, associated with first a decrease and later an entire disappearance of the periodic respirations and periods of unconsciousness, as well as the fall following the relief of the intracranial pressure, are of much interest in connection with the experimental work, and accord so well with the experimental results that further comment is unnecessary.

*Case II.*—I was enabled to make a single observation upon a second patient, Mrs. M., white, aged 76 years, through the courtesy of Dr. Arthur Hebb, in whose service the case occurred, and of Dr. Cushing, who was called in consultation. Two weeks previous to the time when the patient was seen by me, she had suffered from cerebral hæmorrhage. Since that time, she had had Cheyne-Stokes respiration at various times, interspersed with periods of regular respiration. When seen by me, on March 2, 1906, she had well marked Cheyne-Stokes respiration. The left eye showed slight exophthalmus, some œdema of the conjunctiva, and choked disc of moderate extent. The patient was unconscious both in the apnœic and dyspnœic periods. During apnœa, the patient was as a rule quiet, while during dyspnœa, and specially at its height, muscular movements were common. The left pupil was slightly larger than the right. Nystagmus was present. Rhythmic dilatation and contraction of the pupils was associated with each respiration, dilatation occurring shortly after the beginning of each inspiration, and being more pronounced the deeper the inspiration; contraction to the usual size occurred with the following expiration. There was rotation outward and upward of eyes at the beginning of apnœa. No definite difference in size of the pupil in apnœa and in dyspnœa could be made out.

The records reproduced, Tracings 13 and 14, Plate XXXV, show similar relations of blood pressure and respiratory activity as in the last case, namely a rise of blood pressure associated with each respiratory group. The groups of respirations are somewhat more regular than in the first case.

Blood pressure waves rising and falling above and below the line of intracranial pressure were always associated in my experiments with periodic respiration. If this condition changed, the periodicity of the respirations disappeared. The change might consist in lowering of the intracranial pressure so that it was at all times below the blood pressure wave, or in a rise of the blood pressure so that it was constantly above the intracranial pressure, or it might be due to a disappearance of the Traube-Hering waves themselves. Lowering the intracranial pressure to zero results as a rule in the disappearance of the Traube-Hering waves as well as of the periodic respiration, but in a few cases observed, these waves still continued after thus relieving the compression. The periodicity of the respirations, however, at once disappeared, and hence cannot be due in these experiments to the presence of such waves alone. In rare cases, one or two groups of respirations may be observed after lowering the intracranial pressure to zero. Their immediate disappearance is, however, under such conditions a very striking and almost constant fact, and the rare exceptions, in which after one or two groups of periodic movements the respirations become entirely regular, are in all probability to be explained by the assumption that the tension is not immediately relieved in these cases by lowering the pressure bulb. In long experiments, the brain becomes quite œdematous, and the changes in pressure over the medulla when the fluid is allowed to enter over the cortex may not immediately follow the changes in pressure of the entering fluid. Such a result has never been observed when the fluid was allowed to enter directly over the medulla.

It is important to consider the fact that, with intracranial pressure increased to a point near the arterial blood pressure, we have conditions present which if this blood pressure varies may give, so far as the cerebral and medullary circulation is concerned, a sharply marked line between anæmia and a blood supply more or less closely approaching the normal. If the intracranial pressure is greater than the systolic blood pressure, as the result either of a rise of the former or a fall of the latter, the medulla and great brain must be in a state of absolute anæmia, for, the pressure without the capillaries being greater than that within, these vessels must fall into a condition of collapse. A rise of systolic blood pressure above the line of intracranial pressure is coincident with a renewed circulation in these vessels, and the amount of blood forced in with each pulse wave varies directly with the extent of this rise above the intracranial pressure. If, however, the diastolic pressure has not reached the intracranial pressure, during the whole of diastole and a portion of each systole, the vessels are collapsed. If the diastolic pressure now rises above this line, the vessels are not completely collapsed at any time. It is thus apparent that with a gradual rise of blood pressure from a point below the line of intracranial pressure to a point above this line, the blood supply to the cerebral vessels increases at a very rapid rate. Even after the diastolic pressure has risen above this line a further rise of pressure is still associated with a very rapid increase of blood supply, for, according to Poiseuille's law,[14] as the calibre of the capillaries is increased, the blood flow increases by the fourth power. A slight rise of blood pressure, if blood pressure rises from a point equal to or below the line of intracranial pressure, causes a very much greater and more rapid increase in the amount of blood flowing to the medullary centres than would occur with an equal rise of blood pressure under other conditions. Conversely, a similar rapid and great decrease of blood supply occurs when the blood pressure falls from a point equal to or above the line of intracranial pressure to a point below this line.

The condition that is present, when the blood pressure rises and falls in the form of waves above and below the line of intracranial pressure, is one of alternate anæmia and blood supply to the brain. During a fall of blood pressure below this line the capillaries are collapsed during a part or the whole of each systole, or during the whole of both systole and diastole. The former gives a condition of anæmia which varies with the extent of this fall, but must be always considerable. In the latter condition there is absolute anæmia. As the systolic blood pressure rises above the line of intracranial tension, more and more blood enters the cerebral vessels at each systole of the heart, and as the diastolic pressure rises above this line, the blood supply suddenly increases at a very rapid rate. The blood supply to the medullary centres will vary in degree with the extent of this rise of blood pressure above the line of intracranial pressure. With the succeeding fall to a point below the line of intracranial pressure, the blood supply diminishes with great rapidity, and a sudden great reduction occurs as the diastolic pressure falls below this line. It is therefore probable that the amount of blood supply to the medullary centres during the course of a blood pressure wave, which in one part of its curve is below, at another, above the line of compression, varies through a great extent, and these variations occur with great rapidity.

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[14] *Compt. rend. d. l. Acad. d. Sciences*, 1843, xvi, 60.

The law may be thus stated: $Q = K \frac{Hd^4}{l}$ when K is a constant for each liquid, H is the pressure, d the diameter and l the length of the tubes.

The records obtained from the experiments upon periodic respiration and those from the two clinical cases, show that the periods of respiratory activity accompany the rise of the blood pressure wave above the line of intracranial tension; the periods of apnœa are associated with a fall of the blood pressure below this line. That is, the former occur when the brain is more or less well supplied with blood, the latter are associated with the periods of anæmia. The relations existing between the blood pressure and the intracranial pressure determine at these times the extent of this anæmia and blood supply. The anæmia may be relative or absolute, but is always great. The period of blood supply, even at its maximum, is probably always somewhat less than the normal blood supply, because the blood vessels are constantly under the influence of an outside compressing tension which tends to diminish their calibre. The unusually high blood pressure that prevails in this condition may in part compensate for this, but probably only to a limited extent.

The results of recent work prove almost conclusively that carbon-dioxide is the normal and under ordinary conditions the most efficient stimulus of the respiratory centre. Thus Haldane and Priestly,[15] as the result of a large number of observations, have come to the conclusion that diminished supply of oxygen in man is not capable of affecting the activity of the respiratory centre until the amount of oxygen falls to thirteen per cent. of an atmosphere in the respired air, while carbon-dioxide, if increased to three tenths of one per cent. of an atmosphere, increases the pulmonary ventilation two and a half times. Mosso[16] has recently approached the problem from another point of view. In experiments at high altitudes and under low barometric pressures, produced artificially, he has shown that the reduced pulmonary ventilation which up to a certain point is present under these conditions, is associated with a reduction of the carbon-dioxide as well as of the oxygen of the blood. That the lack of oxygen may, however, under certain conditions act as a respiratory stimulus is, I think, admitted by practically all observers. The question that has been productive of so much work is the relative efficiency of the two stimuli, and at present results seem to point to carbon-dioxide as the more efficient stimulus, and probably the only one active under normal conditions, while lack of oxygen may become efficient under conditions in which its supply to the centre is considerably reduced. It is of course impossible to say in any given experiment when this limit is passed. With periodic respiration, the pulmonary ventilation is probably always less than normal, though the decrease in the number of respirations is often in part compensated for by the increase in depth of the individual respirations. This fact would suggest that possibly the centre is responding under these circumstances both to increase of carbon-dioxide and decrease of oxygen. For convenience of discussion, it will be well to treat both stimuli as probably effective under the circumstances. That resulting from increase of carbon-dioxide is probably always present, that caused by lack of oxyen may or may not be. The real determination of this point, so far as the conditions here considered are concerned, is of no great importance. In these conditions, the processes that evidently call forth the periods of respiratory activity and repose serve to increase or decrease both stimuli.

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[15] *Jour. of Physiol.*, 1905, xxxii, 225.

[16] *Arch. ital de biol.*, 1903, xxxix., 395.

We must recognize two conditions which increase the stimulus to the respiratory centre: (1) an increase in the carbon-dioxide tension and a lowering of the oxygen tension in the blood supply to the centre, and (2) a reduction in this blood supply, its carbon-dioxide and oxygen tension when it reaches the centre being unaltered. Both will act in the same way, namely, by preventing a discharge of the carbon-dioxide contents of the cells of the centre and by decreasing their oxygen supply. The extent to which a reduction of blood supply may act as a stimulus to the centre will vary, moreover, with the relative amounts of carbon-dioxide and the oxygen in the blood.

If the respiratory stimulus as ordinarily stated, namely, an increase in carbon-dioxide and a reduction in oxygen, were all that need be considered, this stimulus would certainly be greatest when the reduction of the blood supply to the centre is at its maximum. Under any condition in which the blood supply to the centre varied through a considerable degree, we should expect respiratory activity to be most marked when the anæmia was greatest, while a cessation of activity, *i.e.* apnœa, if such occurred, should be associated with a reduction of the stimulus consequent upon an increased blood supply. With intracranial tension, when the alternate anæmia and blood supply is produced by a wave of blood pressure which rises and falls above and below the line of intracranial pressure, the opposite condition holds, and it is evident therefore that certain additional factors have to be taken into consideration.

One such factor is the evident necessity of a certain blood supply to the respiratory centre in order for it to remain for any length of time in condition to respond to stimuli. The activity of this centre is evidently more dependent upon a proper blood supply than is that of any other medullary centre. The length of the period of apnœa, which is observed so commonly shortly following a rise of intracranial pressure to a point above blood pressure, is, other things being equal, proportional to the extent of the preliminary fall of blood pressure (as the result of vagus inhibition), and the length of time the blood pressure has remained below the line of intracranial pressure. If such a preliminary fall does not occur, no cessation of respiratory activity may follow. This is illustrated in Tracing 3. Following a rise of intracranial pressure above the systolic blood pressure, if the blood pressure does not rise, but continues to fall, or remains constantly below the line of intracranial tension, absolute apnœa develops, after the ordinary preliminary increase of respirations lasting for a few seconds. If the intracranial pressure is now constantly sustained above the systolic blood pressure, apnœa continues unbroken until death. Such a result may occur at the end of a long experiment, and is probably due to a gradual exhaustion of the vasoconstrictor centre, which has been subjected to abnormal stimulation and an insufficient blood supply for a considerable period of time. It is occasionally observed also from unknown causes, either at the beginning of an experiment or later. Finally it may result from continually raising the intracranial pressure until a great height is reached. The vasomotor centre continues to respond under these conditions until a certain point is reached, representing the maximum of its possible activity. If the intracranial pressure is now carried beyond this point, a rapid loss of control occurs in the vasomotor centre, and the blood pressure gradually falls. Under these conditions, absolute apnœa results and continues until death. Such a result is shown in Tracing 15. Tracing 16 shows that after this condition has persisted for some time, removal of the compression, followed by a renewed supply of blood to the respiratory centre, may result in a partial recovery of the centre, sufficient to enable it to respond to strong stimuli. In this experiment, the animal had been bled, 150 cc. of blood having been drawn one and a half hours previous to the beginning of the experiment, and the height of the blood pressure existing at this time probably represented the maximum activity of the vasoconstrictor centre. The first increase of intracranial pressure was productive of no rise in blood pressure, and apnœa soon developed. After some time, the intracranial pressure was lowered to zero, and artificial respiration was begun shortly afterward. Forty-eight seconds later, and seventy-two seconds after lowering the intracranial pressure to zero, natural respirations began, and nine such deep asphyxic respirations were observed before death. These may be seen in the record as the deep respirations between the smaller artificial respirations. The vasomotor centre did not recover, and death occurred shortly afterward. The renewed blood supply to the respiratory centre, produced by lowering the intracranial tension, enabled it to recover to a sufficient extent to respond to the exceedingly strong stimulus prevailing as a result of the long apnœa and low blood pressure.

That the apnœa consequent upon a rise of intracranial pressure above blood pressure is due to the anæmia that results, and does not arise from any inhibitory process that occurs directly as a result of the compression *per se*, is evident from several facts. In the first place, no such effect is obtained with degrees of compression less than blood pressure. Secondly, the length of the apnœa following a rise of intracranial pressure varies directly with the amount and duration of the preliminary fall in blood pressure as stated above. Thirdly, results are obtained from brain anæmia consequent upon ligation of the cerebral arteries which differ in no way from the results obtained from the anæmia produced by an increase of intracranial pressure above blood pressure. In a number of experiments such ligation was performed, the animals used being dogs and rabbits. The results may be stated as follows:

Ligation of the four cerebral arteries (both carotids and both vertebrals at or near their origin) in dogs is without effect upon respiration, the rate and depth remaining unchanged. The ligation of each is followed by a slight rise of blood pressure which continues at this new level. This rise probably leads to an increase in the blood supply to the medulla through the cervical and spinal anastomoses, sufficient to supply the necessary amount of blood to the respiratory centre. Ligation of three of the cerebral arteries, one carotid or one vertebral remaining free, combined with ligation of the subclavians below the origin of the cervical branches, results in a slight increase in respiratory rate and depth, passing to the normal rate and depth after a variable length of time. With the rise of blood pressure and the enlargement of the spinal anastomoses, the reduction in the blood supply becomes less marked, and there probably also results a slight depression of the irritability of the respiratory centre in consequence of its reduced blood supply; both factors tend to cause a return of the normal rhythm and depth. It seems, therefore, that the cervical and spinal anastomoses in the dog are more important to the blood pressure in the circle of Willis than any one of the four main cerebral arteries. Ligation of the four cerebral arteries, combined with ligation of the subclavians, leads to an increase of respiration, succeeded by a decrease which finally ends in apnœa. This apnœa is attended by the development of vagus inhibition, and a subsequent rise of blood pressure, at the summit of which the apnœa is broken and respiration gradually returns, a series of events identical with that observed when the intracranial pressure is raised to a point equal to arterial blood pressure. It appears from these experiments that the spinal anastomoses in the dog are alone sufficient, when the blood pressure is high, to supply the respiratory centre with a sufficient amount of blood to enable it to respond, while if the pressure is not thus raised, the activity of the respiratory centre soon ceases, the reduced supply of blood lowering the irritability of the centre below the point at which the existing stimulus is effective. Ligation of three cerebral arteries, combined with ligation of the subclavians and of the spinal cord at the first thoracic vertebra, increases temporarily the respiratory rate and depth. No rise of blood pressure is possible because of the ligation of the cord, and the return to normal rate and depth is probably due to a slight decrease in irritability of the respiratory centre. A record of the changes which occur when one vertebral remains unclamped, follows the first mark in Tracing 17. The circulation through one carotid or one vertebral is thus sufficient in the dog for the continued activity of the respiratory centre. Ligation of all of the main cerebral arteries, combined with ligation of the subclavians and the cord, leads to a slight increase in rate and depth of the respirations passing rapidly into a decreasing stage, and apnœa shortly follows, accompanied by vagus inhibition. This condition follows the second mark in Tracing 17. The occurrence of apnœa is much more rapid than when the spinal arteries are not ligated. Removal of the clamps from the two carotids (at third mark), is followed in a few seconds by a return of respirations, at first slow, and then gradually increasing to normal.

The respiratory centre of the rabbit is more susceptible to a decrease in its blood supply than that of the dog, though there is much more individual difference shown. Ligation of one cerebral artery leads in most cases to an increase in rate and depth of the respirations. In two cases ligation of two of the cerebral arteries led to immediate apnœa unbroken until death. As a rule, ligation of from two to four of the cerebral arteries leads to apnœa, with or without preliminary increase of the respirations; apnœa may be absolute or be broken after fifteen seconds or more as a result of the following marked rise in blood pressure and the great increase in the respiratory stimulus caused by the long period of apnœa. Tracings 18 and 19[17] are from an experiment on a rabbit in which the last cerebral artery was clamped. In Tracing 18, the last artery was periodically compressed and released, each compression leading to an immediate rise of blood pressure and apnœa, each release to an immediate fall of blood pressure and recurrence of respiration. In Tracing 19 with the two compressions, the rise of blood pressure associated with the great increase of the stimulus as a result of the long apnœa, was sufficient to stimulate the centre to slow and weak responses while the artery still remained clamped.[18]

A large accumulation of carbon-dioxide and a great reduction of oxygen in the blood, does not for a considerable length of time affect the power of response of the respiratory centre. Thus in ordinary asphyxia from occlusion of the trachea, and to a somewhat less extent in the asphyxia following hæmorrhage, the respiratory centre continues to respond until the condition has led almost to death, and for the greater part of the time with increasing activity to the constantly increasing stimulus. It is not until after the lapse of from twenty to thirty seconds or more that the reduction of irritability of the respiratory centre proceeds more rapidly than the increase in the strength of the stimulus, and the centre begins to show decreasing activity of response. The power of response does not reach zero until the other medullary centres are almost completely paralysed. There is only a moderate reduction in blood supply to the respiratory centre in the case of hæmorrhage, and an increase in the blood supply due to rise of blood pressure for a great part of the time in the asphyxia following ligation of the trachea. The condition is entirely different from the great or even absolute reduction consequent upon the increase of the intracranial tension to equal to or above blood pressure, or consequent upon the occlusion of all or the greater part of the vessels supplying blood to the brain. The difference is sharply defined. In the former there is a diminished, normal or increased blood supply associated with an increase in the strength of the stimulus (more carbon-dioxide and less oxygen) and the result is an augmented activity of the centre. In the latter, the increase in the respiratory stimulus may be supposed to be as great or even greater, but it is associated with a marked reduction in or an absolute deprivation of the normal blood supply. The result is a rapid or immediate cessation of the activity of the centre. It is evident, therefore, that when the blood supply to the respiratory centre falls below a certain low point, the irritability of the centre is so reduced that it is not able to respond to the strongest stimulation. A somewhat less reduction of blood supply lowers its irritability so that it is only capable of response to very strong stimuli. A considerable reduction of blood supply is necessary to perceptibly change the irritability of the centre; a sufficient reduction results in a rapid decrease of the irritability, but a renewed supply causes rapid recovery unless anæmia has been too long continued.

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[17] In the experiments on rabbits the respirations were recorded from a slip of the diaphragm by a method similar to that suggested by Head (*Jour. of Physiol.* 1889, xi), and the up-stroke represents inspiration. In the experiments on dogs a transmitting tambour was employed, and the downstroke represents inspiration.

[18] The respiratory muscles exhibit marked changes in tonicity under these conditions. Similar changes, though as a rule not so marked, are frequently observed in dogs in the period of apnœa following an increase of intracranial tension. These changes consist in an inspiratory spasm of short duration, followed by relaxation in the expiratory phase.

*It is thus evident, that in an explanation of periodic respiration which has as its underlying cause a periodic alternation of great or absolute anæmia, with a blood supply to the respiratory centre, more or less closely approaching the normal, a new factor has to be taken into account—namely, the variations in irritability of the respiratory centre as a result of the changes in its blood supply.*

Depending upon the amount of reduction of this irritability, a greater or less interval of time, after the return of blood supply to the centre, will be necessary for its irritability to be raised to a point at which the stimulus becomes effective. Thus if the centre has been subjected to an anæmia of considerable length and great extent, as for example by a long continued increase of intracranial pressure above blood pressure, the lowering of the intracranial pressure to zero and the return of blood flow to the vessels of the centre is not at once followed by a response of the centre, but a longer or shorter time elapses before the irritability of the centre is so raised in consequence of the renewed blood supply that it may be acted upon by the stimulus. On the other hand, if this previous anæmia has not been maintained so long, a return of blood supply to the centre may be coincident with a renewal of its activity. With still less intense grades of anæmia, the irritability of the respiratory centre is so reduced that it is unaffected by the existing stimulus and apnœa results, but it is not so greatly reduced that it is incapable of responding to very strong stimuli. A consideration of these facts in connection with the observations recorded in the two preceding sections, will, I think, make evident their importance in interpreting the results of the records from the experiments and from the clinical cases. It was there noted that the groups of respirations may precede by a short interval, accompany, or follow the rise in blood pressure. They follow the rise of blood pressure especially with the long periods of apnœa which are associated with long periods of anæmia, the condition in other words that gives rise to marked depression in the irritability of the centre.

A suddenly increased supply of blood to a respiratory centre which has its irritability considerably depressed as the result of long continued insufficient blood supply, but is nevertheless still capable of response, may result in apnœa. This condition has been observed a number of times. Following a rise of intracranial pressure, if the blood pressure also rises and remains constantly above the former, respirations soon recur and continue regularly. Frequently the blood pressure is only slightly sustained above the intracranial pressure under such conditions, and the diastolic pressure may be even below this line, so that only a portion of each pulse wave finds its way into the cerebral vessels. If, however, no variations of blood supply occur, respiratory activity is regular in force and rhythm. At first it may be somewhat increased, but after a short time it usually continues uniformly at a rate which is considerably less than normal, and without increased depth. In consequence of the considerable reduction of the blood supply, the stimulus must be considerably increased, and the lessened activity of the centre is an expression of its depressed irritability in consequence of its reduced blood supply. If under such conditions the intracranial pressure is suddenly lowered, the activity of the respiratory centre may at once cease, and apnœa result. If the intracranial pressure is now suddenly raised again to its former height, an immediate return of respirations will occur. If it is not thus again raised, respiratory activity returns after a longer or shorter apnœa and rapidly increases to an extent greater than that which was present before the intracranial pressure was lowered, *i.e.*, to an extent equalling or approaching the normal activity. The most probable explanation of this result is to be found in the fact that the sudden and great flow of aerated blood to a centre the irritability of which has been considerably decreased as the result of anæmia, suddenly lowers the carbon-dioxide tension of the cells of the centre and at the same time increases their oxygen supply. The stimulus is thus reduced, and the reduction no doubt occurs with great rapidity because the blood is fairly well aerated. The greater flow of blood increases the irritability, but probably not so rapidly as it reduces the stimulus. After a period of apnœa, during which the irritability of the centre and the strength of the stimulus gradually increases, respiratory activity recurs, and is greater than before because of the improvement in the irritability of the centre. Apnœa may be terminated at any time by a sudden increase of the stimulus as a result of raising the intracranial pressure. The phenomenon just described is only observed when the centre is considerably depressed, and has never been observed at the beginning of an experiment or when the centre was responding vigorously and showed no evidence of a reduction in its activities. In the majority of cases, lowering the intracranial pressure when the centre is responding, does not result in apnœa, but a gradual improvement in rate and depth which begins almost immediately.

A rise of blood pressure above the line of intracranial pressure is similar in every way, so far as the cerebral circulation is concerned, to a fall of the latter below the blood pressure. With considerable depression of the respiratory centre as a result of long continued anæmia, a return of blood to the centre may not result in an immediate respiratory response. This is the condition observed especially in periodic respiration with long periods of apnœa. Here almost invariably, a rise of blood pressure above the line of intracranial pressure precedes for some time the beginning of respiratory activity. As a result of the long continued periods of anæmia associated with the long intervals of apnœa, the respiratory centre is greatly depressed, and some interval after the return of blood supply is necessary for its irritability to be raised to a point sufficient for the stimulus to become effective.

*It is thus evident that in a condition of alternate anæmia and blood supply to the respiratory centre, there are two factors acting in opposite directions. A renewed supply of blood to the centre, following upon a period of anæmia, results in an increase in the irritability of the centre, and at the same time decreases the stimulus to the centre. The succeeding fall of blood pressure and production of anæmia, results in an increase of the stimulus and a reduction of irritability.* Which process will gain the upper hand and determine the result as regards the respiration, depends upon the condition of the centre, the rapidity of the change, and the gaseous composition of the blood. It is impossible of course to know all of these conditions at any one time, and inferences concerning them must be drawn in large part from the processes themselves.

The foregoing discussion and experiments have clearly shown, I think, how remarkably susceptible the irritability of the respiratory centre is to a considerable reduction of its blood supply. This peculiarity is not shared by the other medullary centres. Thus the vasomotor centre may, and frequently does show its first response to stimulus when its blood supply is entirely cut off. After ligation of the cerebral arteries the blood pressure rises markedly and continues to rise many seconds after the respiratory activity has been completely stopped as a result of the anæmia. The rise of blood pressure that results after raising the intracranial pressure above blood pressure, frequently begins when the blood pressure is extremely low, owing to simultaneous inhibition of the heart, and it may be a number of seconds before the rise of blood pressure to a point above the intracranial pressure results in a renewed supply of blood to the vasomotor centre. Furthermore, it is evident that accumulation of carbon-dioxide and lack of oxygen in the blood may act as a very efficient stimulus to the vasomotor centre. This is readily observed of course in ordinary asphyxia.

With increased intracranial tension Traube-Hering waves are evidently due to a periodic increase and decrease of the stimulus to the vasomotor centre, the increased stimulus being coincident with an anæmia of the centre. Under normal conditions it is probable that the irritability of the vasomotor centre to excess of carbon-dioxide and lack of oxygen is very much less than the irritability of the respiratory centre to such stimuli. Thus, the comparatively minute changes of these gases in the blood under normal conditions, is sufficient to stimulate the respiratory centre to a rhythmic discharge, while they are probably entirely without effect upon the vasomotor centre. The only changes in blood pressure under normal conditions are the respiratory waves, representing a slight rise and fall with each respiratory act. These waves may be accounted for entirely by mechanical effects, including in this term the changes in pressure in the thorax, together with change in the heart rate as a result of periodic vagus action. It is probable that under normal conditions the most efficient stimulus to the vasomotor centre and the one that keeps it in constant tone, arises from stimulation of the various sensory nerves.

As regards the comparative activities of the respiratory and vasomotor centres, I think we are justified in drawing the following conclusions:

(1) The respiratory centre is normally more susceptible to slight changes in carbon-dioxide and oxygen than is the vasomotor centre.

(2) The normal irritability of the respiratory centre is more dependent upon a proper blood supply than is the irritability of the vasomotor centre. The irritability of the former centre may be rapidly reduced by anæmia and rapidly increased again as the anæmia passes away.

(3) The vasomotor centre may respond to stimuli after many seconds of absolute anæmia.

(4) By the action of anæmia, the normal relations may readily and quickly become reversed, and the vasomotor centre become more susceptible to the existing stimulus than the respiratory centre.

It appears that the cardio-inhibitory centre may be likewise efficiently stimulated by the gaseous changes in the blood. After a rise of intracranial pressure, the vagus inhibition does not develop as a rule until the intracranial pressure reaches arterial pressure, and it passes off to a great extent with the following rise of blood pressure above the intracranial pressure, though it nearly always persists to a certain degree, the pulse rate being slower throughout the compression than before. With periodic respiration associated with Traube-Hering waves, inhibition is removed in part as the blood pressure rises above the intracranial pressure, to return as it falls below the intracranial pressure during the following period of apnœa. After section of both vagi the pulse rate is the same in the periods of apnœa and respiratory activity (Tracing 5). During periodic respiration with intact vagi, each respiration of the group is followed by a rapid removal of vagus inhibition occurring almost coincidently with the respiration, and then the pulse becomes progressively slower until the occurrence of the next respiration. Tracing 20, taken during periodic respiration, shows that this removal of vagus inhibition occurs very rapidly upon the recurrence of the respiration, following the beginning of inspiration in one quarter of a second or less. It is apparently too rapid to be a gaseous effect, and is probably an exaggerated example of the phenomenon which occurs with each inspiration under normal conditions, and according to Fredericque is due to associated activity of the respiratory and cardio-inhibitory centres. The extent of this removal of vagus inhibition in conditions of increased intracranial tension varies greatly at different times and is independent of the depth of inspiration. At one time a large respiration may call forth only slight removal of vagus inhibition, while a much smaller one is attended with correspondingly great removal of vagus inhibition. The conditions which underlie this difference are not as yet clear, and much work will have to be done before the vagus effects in intracranial tension are well understood. The important point that I wish to emphasize now is that the rise of blood pressure above the level of intracranial pressure and the occurrence of respirations in periodic respiration are associated with the partial removal of vagus inhibition, which returns as the blood pressure subsequently falls below the intracranial pressure. Two distinct factors aid in this result: (1) One factor is the rise of the blood pressure above the intracranial pressure line and may be regarded as independent of respiratory activity. Partial removal of the stimulus to the cardio-inhibitory centre probably occurs because of the increased blood supply. It may be observed to occur in certain instances when the blood-pressure wave in its rise above the line of intracranial pressure does not call forth respiratory activity and as well in such waves before the group of respirations has commenced. (2) Each respiration of the group is associated with a rapid removal of vagus inhibition. This effect is too rapid to be due to a gaseous change, and is probably the result of an associated activity of the centres. The partial removal of vagus inhibition constitutes an important factor in the rise of blood pressure associated with each respiratory group in periodic respiration. Both of the causes of removal of vagus inhibition above described are, I think, clearly brought out in the tracings which are reproduced.

### CHEYNE-STOKES RESPIRATION OCCURRING CLINICALLY IN CONDITIONS OTHER THAN THAT OF INCREASED INTRACRANIAL TENSION.

Continuous records of blood pressure in association with periodic respiratory phases have been obtained in eight such cases. Two of these were cases of myocarditis and generalized arteriosclerosis, one of myocarditis and mitral insufficiency, two of myocarditis and aortic insufficiency, one of aortic aneurism associated with aortic insufficiency, one of nephritis with marked cardiac hypertrophy and insufficiency, and one of nephritis with uræmic symptoms. Changes in blood pressure recorded by the tracings were constant in all these cases and were as follows: During apnœa, usually about the middle of the period, a fall of blood pressure begins, gradually increases, and is associated with a slowing of the pulse which develops gradually. Both fall of blood pressure and slowing of the pulse continue to the onset of respiratory activity and reach their maximum at about the third or fourth respiration of the group. A gradual rise of blood pressure and a gradual augmentation in pulse rate begin from this point and increase throughout the remainder of the respiratory period and through approximately the first half of the following interval of apnœa. This rise in blood pressure is at first slow, but toward the end of dyspnœa and the beginning of the following apnœa it is more marked.

It is thus apparent that the relations of blood pressure and respiratory activity which prevail here are almost the exact opposite of those observed with increased intracranial tension. The periods of apnœa are associated with an increased blood pressure, the periods of respiratory activity with a fall of blood pressure. The relation of the pulse rate to the blood pressure is the same that is found with the cases of increased intracranial pressure and in the experiments, the slower rate being associated with the lower blood pressure.

Tracings 21 and 22 show the condition described above. This type of Cheyne-Stokes respiration is being investigated by the author and will form the subject of a subsequent paper. Only certain points will be referred to here.

The groups of respirations consist in nearly all cases of a definite ascending and descending phase, and markedly atypical groups such as those commonly occurring in cases with increased intracranial pressure have not been observed. The relative lengths of the apnœic and dyspnœic periods are also more constant.

Disappearance of the periodicity of the respirations is accompanied by a disappearance of the blood pressure waves. The extent of the blood pressure bears a relation to the extent of the variations of respiratory activity. Tracings 23, A, B, and C, were obtained during the gradual decrease in the intensity of the periodic respiratory activity. Tracing 23, A shows the most marked variations in respiratory activity with the most marked blood pressure changes. Tracing 23, B, taken forty-five minutes later from the same patient, shows less marked respiratory variations and less marked blood pressure changes. Tracing 23, C, was taken three hours later, and shows an almost complete disappearance of the periodicity of the respirations with barely perceptible blood pressure changes. The periodicity of the respirations is often more marked when the patient is asleep or quiet; records taken from the same patient during waking and sleeping within a very short period of time (several minutes) have clearly demonstrated this fact.

The periods of respiratory activity are nearly always dyspnœic in character and the patients complain of shortness of breath. The periods of apnœa are associated with quiet and rest, in some cases with partial unconsciousness, but in none of the cases was unconsciousness so deep that the patient could not be readily aroused. In several cases long periods of apnœa were accompanied by no impairment of consciousness. This is in marked contrast to the condition observed in the cases of increased intracranial pressure.

Examination of the eye grounds shows that the changes in blood contents of the retinal vessels correspond to the changes in blood pressure as observed in the brachial artery. Dr. Bordeley was kind enough to make this examination for me, and was able to state positively that the retinal artery and its branches contained more blood at the middle of apnœa than at the height of dyspnœa.

The changes in blood pressure in this group of cases of Cheyne-Stokes respiration, as well as in the cases of increased intracranial pressure, cannot be merely secondary mechanical effects of the periodic respiratory activity. Tracing 24 is the record of an experiment in which a normal subject breathed periodically and endeavored to simulate Cheyne-Stokes respiration as closely as possible. A number of such records were obtained, and the fact that the conditions observed were absolutely constant in all will exclude, I believe, the possibility that they are due to psychic influences other than those that would be constant under the circumstances. The conditions constantly observed are: (1) A constant blood pressure, or a slight rise throughout apnœa continuing up to the first respiration of the group. (2) A sudden fall of pressure associated with slight and temporary slowing of the pulse, coincident with the first respiration of the group, and followed by a rapid rise to the usual height. (3) A constant or slightly falling pressure throughout the respiratory group, with a sudden fall of pressure toward the end of the group, associated with a marked increase in pulse rate and a sensation of syncope. This fall in turn is followed by a rapid rise to normal or to a level slightly above normal.

## THEORY OF CHEYNE-STOKES RESPIRATION WITH INCREASED INTRACRANIAL TENSION.

In view of the facts brought out by the study of periodic respiration produced experimentally and by observation of clinical cases, in connection with theoretical considerations and changes following ligation of the cerebral arteries, it seems to me that periodic respiration with increased intracranial tension has as its underlying cause a periodic alternation of anæmia, and blood supply to the medullary centres.

In describing the tracings from the experiments and the clinical cases, I have mentioned that the groups of respirations may follow, occur coincidentally with, or slightly precede the beginning of the rise of the blood pressure wave. This circumstance allows these cases to fall naturally into three groups, which will be here considered.

In the first group, the beginning of respiratory activity is preceded by the rise of the blood-pressure wave. It is probable that during the period of anæmia, the irritability of the respiratory centre is so lowered that it is not able to respond to the existing stimulus, nor probably in most cases to any stimulus, no matter how great. The vasoconstrictor centre is finally stimulated to activity by the great increase of the stimulus; and the blood pressure begins to rise. If this rise is sufficient, there may result a partial removal of vagus inhibition, which aids in elevating the blood pressure. As the systolic pressure rises above the intracranial pressure, more and more blood reaches the respiratory centre. This has two effects, (1) to improve the responding power of the centre by increasing its irritability, and (2) to decrease the stimulus to the centre by supplying more blood to it. The latter effect is probably not now very great, as the blood which is supplied is very rich in carbon-dioxide and poor in oxygen, as a result of the long apnœa, and probably does not change the tension of these gases surrounding the centre to any considerable extent. The irritability is thus probably increased more rapidly than the stimulus is decreased. The first respiration leads to a marked removal of vagus inhibition, and the pressure rises still further. This rise of pressure supplies the respiratory centre with more blood, its irritability is still further raised and it responds with increased activity. Each respiration is usually preceded by a slight fall of blood pressure which slightly increases the stimulus. Soon the effect of the respiratory activity upon the gaseous condition of the blood flowing to the respiratory centre is felt. The blood is no longer loaded with carbon-dioxide and poor in oxygen, but is more or less well aerated. The stimulus arising from this increased supply of well aerated blood falls within the threshold of irritability of the respiratory centre in its improved condition, and the increasing scale of respiration ceases. The increased supply of well aerated blood to the vasoconstrictor centre also causes it to relax, and this relaxation, with the increased vagus inhibition, leads to a fall of blood pressure. This fall leads to two conditions: (1) a decrease in the irritability of the respiratory centre, and (2) an increase in the stimulus. The decrease in irritability is probably considerable, while the increase of stimulus is not marked, because it is caused by a reduced supply of blood that is well oxygenated and poor in carbon-dioxide, as a result of the respiratory activity. The former factor exceeds the latter, and with such a fall of the irritability that the stimulus becomes inactive, apnœa begins. The decreasing group of respirations is an expression of the fact that although the stimulus is increasing, as it must under these circumstances, the irritability of the respiratory centre is being more rapidly lowered. To explain the rise of blood pressure, we assume that the irritability increases more rapidly than the stimulus decreases, because the blood is poor in oxygen and rich in carbon-dioxide, while with the fall of blood pressure the inverse relations hold because of the opposite condition of the gases in the blood. All the experimental evidence tends to show that the supply of blood, in large part independently of its relative richness in oxygen and in carbon-dioxide, is the important factor in determining the irritability of the respiratory centre.

In the third group of cases, where the beginning of respiratory activity precedes slightly the rise of the blood pressure wave, we assume that the conditions are more nearly normal; the irritability of the respiratory centre, though reduced, is still greater than that of the vasoconstrictor centre, and the accumulation of carbon-dioxide and decrease of oxygen will stimulate the former first. The greatly increased stimulus arising from the great increase in carbon-dioxide and decrease in oxygen in the reduced blood supply to the respiratory centre finally becomes sufficient to excite it to activity. The respiratory centre then finally responds, at first very feebly. The first one or two respirations have little effect upon the stimulus to the vasoconstrictor centre because of its greatly reduced blood supply. It soon becomes stimulated to activity. If a rise of blood pressure did not occur, the respirations would soon cease, owing to the slight reduction of the stimulus to the respiratory centre as the result of the aeration of its diminished blood supply and the absence of any condition which would tend to raise its irritability. The first respiration usually raises slightly the blood pressure in consequence of the partial removal of vagus inhibition, but this rise of pressure is usually not marked, owing probably to the small size of the respiration, and to the fact that one factor which favors removal of vagus inhibition, namely, the occurrence of a supply of aerated blood, is under the circumstances very insignificant. If the first respiration is large, and the anæmia not very marked, the rise of pressure may be greater. With the rise of the blood pressure consequent upon vasoconstrictor stimulation, the further course is similar to that in the first group of cases. In the third group of cases there are relatively short periods of apnœa and relatively long periods of respiratory activity. The blood pressure does not as a rule fall to such an extent during the periods of apnœa as in the first group, and the periods of apnœa are probably never associated with absolute anæmia. The inverse relations hold in the first group described.

In the second group of cases, the rise of pressure and beginning of respiratory activity are coincident. The condition of the centres and the changes of blood pressure and respiration are to be regarded here as midway between those present in the first group and those present in the third group. The irritability of the respiratory centre has been reduced to a point equal to that of the vasoconstrictor centre. The changes which occur after beginning of respiratory activity are identical with those in the other two groups of cases.

Corresponding with variations of the blood-pressure wave, the groups of respirations may assume various forms besides the typical one described above. As already noted, these atypical groups were unusually common in the clinical cases. With a rapid sudden preliminary rise of blood pressure, the blood supply to the respiratory centre is rapidly increased, and the resultant increase of irritability of the respiratory centre may be sufficient to enable it to give a large first response. This may be sufficient to relieve the stimulus to the vasomotor centre, so that a rapid fall then commences, and thus a decreasing group results. If the pressure continues to rise, the succeeding respirations will probably be larger. If the fall occurs more slowly, because the slightly increased stimulus resulting from the fall is not exceeded by the depression of the irritability of the centre, a group of equal respirations may result, the respiratory activity being finally terminated by a more sudden fall. In the experimental cases, the fall of blood pressure following the last respiration of such a group is very sudden and great, usually dropping almost immediately to some distance below the line of intracranial tension. A sudden fall of pressure occurring at the height of a gradually increasing group may terminate the respiratory activity at once and prevent the descending phase. The cause of such a fall of blood pressure is evidently the decrease in the stimulus to below the threshold of irritability of the vasoconstrictor centre. The irritability of this centre is dependent both on the supply of blood to the centre and on the preceding respiratory activity; the latter probably determines in large part whether the fall of pressure occurs slowly or more rapidly.

## CONCLUSIONS.

In addition to the results brought out in the separate sections I wish to direct especial attention to the following conclusions:

(1) In ten cases of Cheyne-Stokes respiration observed clinically, the alternate periods of respiratory activity and apnœa were associated with Traube-Hering waves of blood pressure. These cases may be separated into two groups characterized by the relation of the respiratory changes to the changes of blood pressure. In one group the period of respiratory activity was associated with a rise of blood pressure, the period of apnœa with a fall; in the other group, the reverse relations existed. The former group included two cases of Cheyne-Stokes respiration

occurring with increased intracranial tension; the latter contained eight cases with cardiac and arterial disease.

(2) By means of cerebral compression, periodic respirations may be produced experimentally, and the relation of the blood pressure changes to the respiratory variations are the same as in the clinical cases with increased intracranial tension, namely, a rise of pressure with each group of respirations and a fall with each period of apnœa. In the experiments, during each respiratory group, the blood pressure rises above the line of intracranial tension and with each period of apnœa it falls below this line. With the disappearance of this relation, the periodicity of the respirations likewise disappears. It is probable that the same relation between the blood pressure and intracranial pressure exists when Cheyne-Stokes respiration occurs clinically in association with increased intracranial tension.

(3) Disappearance of the periodic respiratory activity in the clinical cases of both groups is accompanied by disappearance of the waves of blood pressure.

(4) The waves of blood pressure cannot be regarded as a mechanical effect of the periodic respiratory activity; on the contrary the latter must be due to the changes of blood pressure, or both phenomena may be referable to a common cause.

(5) Cheyne-Stokes respiration in states of increased intracranial tension, with blood pressure waves rising and falling above and below the line of intracranial tension, is due to periodic activity of the respiratory, vasomotor, and cardio-inhibitory centres, the underlying cause of which is an alternate anæmia and blood supply to the medullary centres. The vasomotor centre, as the result of periodic increase and decrease of the stimulus, shows periodic variations in its activity. It is stimulated to greater activity during the periods of anæmia, and partially relaxes with each period of blood supply. During the periods of anæmia, the respiratory centre loses its irritability for the acting stimulus, and is therefore apnœic. It is finally stimulated to activity, either as a result of an increase in its irritability from a preceding rise of blood pressure, or from a great increase in the respiratory stimulus. The cardio-inhibitory centre is stim-

ulated by the periods of anæmia. This stimulation causes slowing of the pulse, which passes off to a considerable extent with the following period of blood supply.

(6) Cheyne-Stokes respiration has heretofore been regarded as always the manifestation of the same conditions and capable of the same explanation; the results of this work, on the contrary, show that two distinct groups of cases may be recognized, depending upon the relation of the blood pressure changes to the periodic respiratory activity.[19]

(7) The medullary centres show great differences in their susceptibility to anæmia. The respiratory centre is very susceptible to a much reduced blood supply; its irritability is rapidly reduced or lost upon the occurrence of marked or complete anæmia, and is rapidly regained when the blood supply is renewed if the anæmia has not been maintained too long. The effect of a considerable anæmia upon this centre is entirely different from that of a normal or somewhat reduced supply of blood which is more venous than normal, that is, contains more carbon dioxide and less oxygen, such as occurs with ordinary asphyxia. The vasomotor and cardio-inhibitory centres are not nearly so susceptible to anæmia. The former centre may, and frequently does, respond when in a condition of complete anæmia.

(8) I am able to confirm, as a result of my experiments upon cerebral compression, in all essential details the conclusions of Cushing, and the general law formulated by him, namely that "an increase of intracranial tension occasions a rise of blood pressure which tends to find a level slightly above that of the pressure exerted against the medulla."

The author desires to express his indebtedness to Prof. W. H.

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[19] It is interesting to note in this connection that Mosso, in a recent publication of tracings obtained in periodic respiration occurring in normal men at high altitudes ("Fisiologia dell' uomo sulle Alpi," Figs. 31 and 32, and "La resp. period. telle qu' elle se produit chez l' homme sur les Alpes par l' effet de l' acapnie," *Archiv. ital. de biol.*, 1905, xliii, Fig. 21), has depicted what constitutes apparently a third group. Under these circumstances, the rise of blood pressure which occurs with each respiratory group is accompanied by slowing of the pulse. This form of periodic respiration differs therefore from both of the groups described by me.

Howell, under whose direction the work was performed, for invaluable aid and criticism, and to Dr. Harvey Cushing for suggestions and assistance. His thanks are also due to the other members of the Physiological Department and of the staff of the Johns Hopkins Hospital for assistance and clinical facilities.

## EXPLANATION OF PLATES XXXI–XL.

Tracings 1, 2, and 3. Periodic respiration in a dog as the result of an increase in the intracranial tension. The arrangement of the curves is explained in the text.

Tracing 4. Periodic respiration in a dog as the result of an increase in the intracranial tension after the section of one vagus. See text.

Tracing 5. Periodic respiration in a dog as the result of an increase in the intracranial tension after section of both vagi. The arrangement is the same as in Tracing 4 (see text) except that the base line is not shown.

Tracings 6–11. Records from a clinical case of periodic respiration occurring in association with increased intracranial tension. See explanation of curves in the text.

Tracing 12. Blood pressure tracing obtained from the same case as the preceding records, at a time when periodic respiration was not present.

Tracings 13 and 14. Similar tracings from a second clinical case of periodic respiration occurring in association with increased intracranial tension.

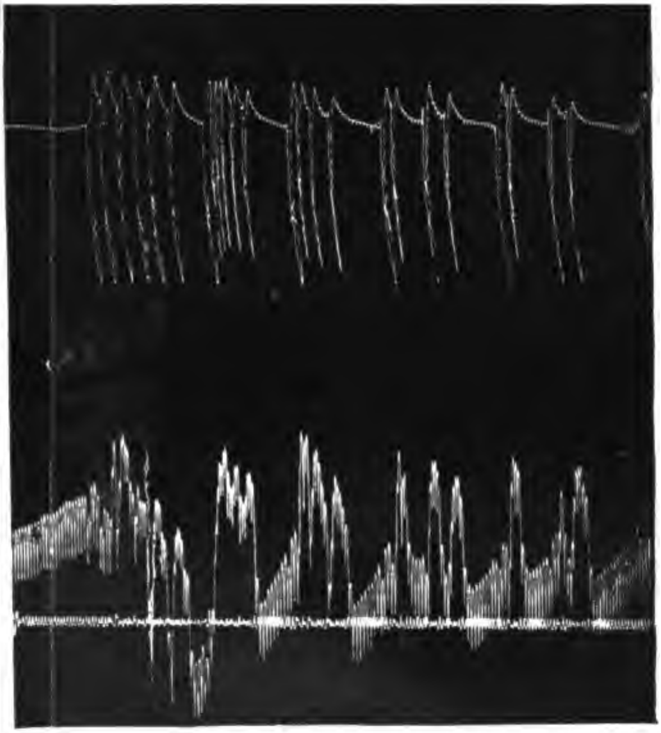
Tracing 15. Record of death of a dog from a great increase in intracranial tension. The uppermost line records the time in two-second intervals. The second curve is the respiratory record, the down stroke representing inspiration. The intracranial pressure is represented by the line that is raised from zero in the first part of the record. The lowermost line is the base line for both the intracranial and blood pressures. The blood pressure was obtained from the carotid artery. This and subsequent records read from left to right.

Tracing 16. Shows the return of natural respiration after lowering the intracranial pressure to zero in an experiment in which the increase of intracranial pressure was not followed by increase in blood pressure. The arrangement of the curves is the same as in the last tracing. The nine deep excursions of the respiratory pen toward the end of the curve represent natural respiration, the smaller excursions are artificial respirations.

Tracing 17. Ligation of cerebral arteries in a dog. The uppermost curve is the respiratory tracing; the down strokes representing inspiration. The second is the blood pressure from the carotid artery. The third line is the base line, the fourth the time in two-second intervals. See text.

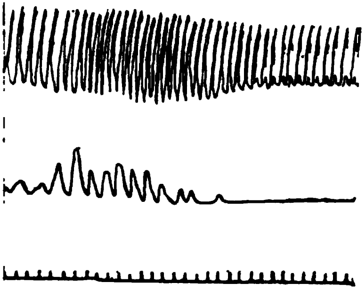
Tracings 18 and 19. Ligation of cerebral arteries in a rabbit. The upper line is the respiratory tracing, the upstroke representing inspiration. The lower line is the blood pressure from the carotid artery. The two vertebrals and one carotid were ligated, and the other carotid alternately clamped and released. The occlusion of the artery is associated with a rise in blood pressure, its release with a fall. In Tracing 19 the release of the artery is noted by "off."

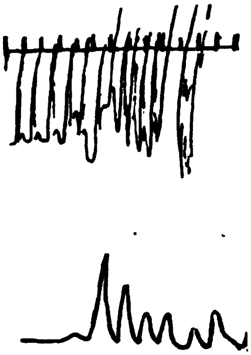
PLATE XXXI.



Tracing 2.

## PLATE XXXIV.





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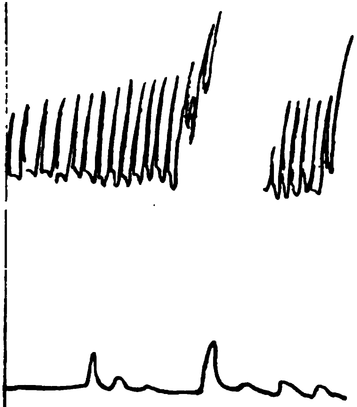

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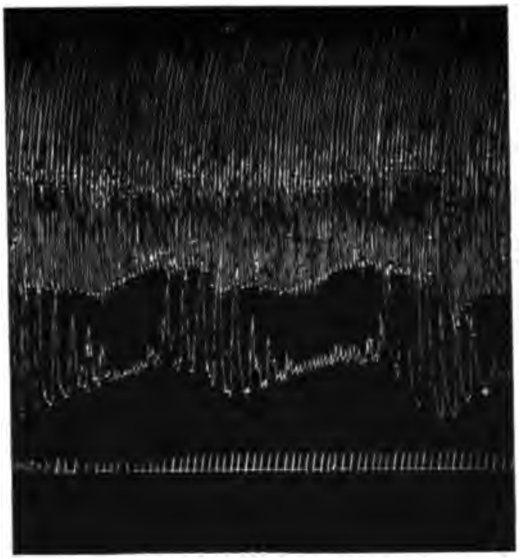

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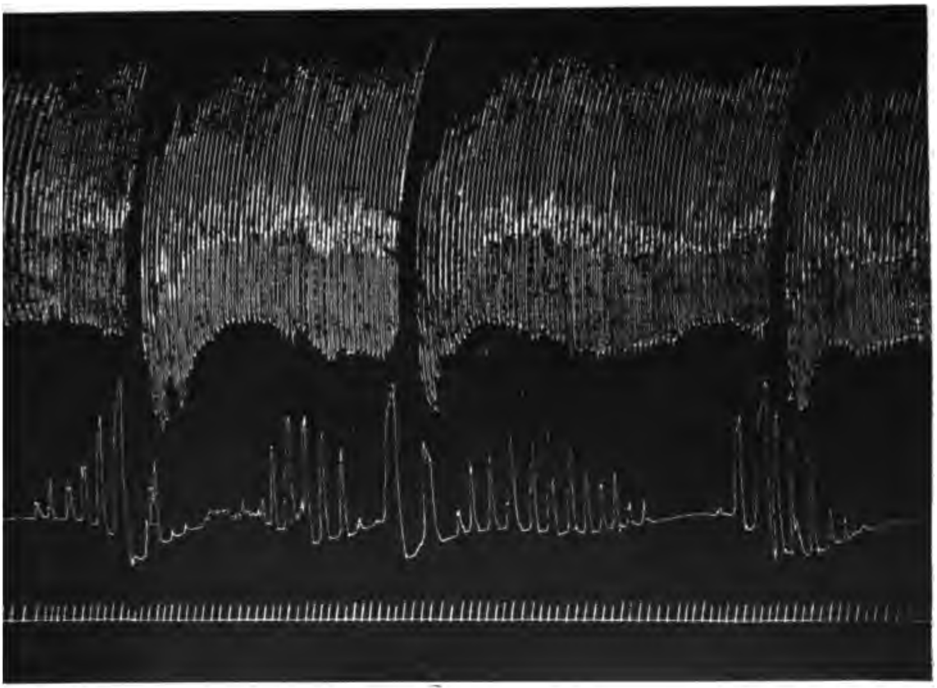

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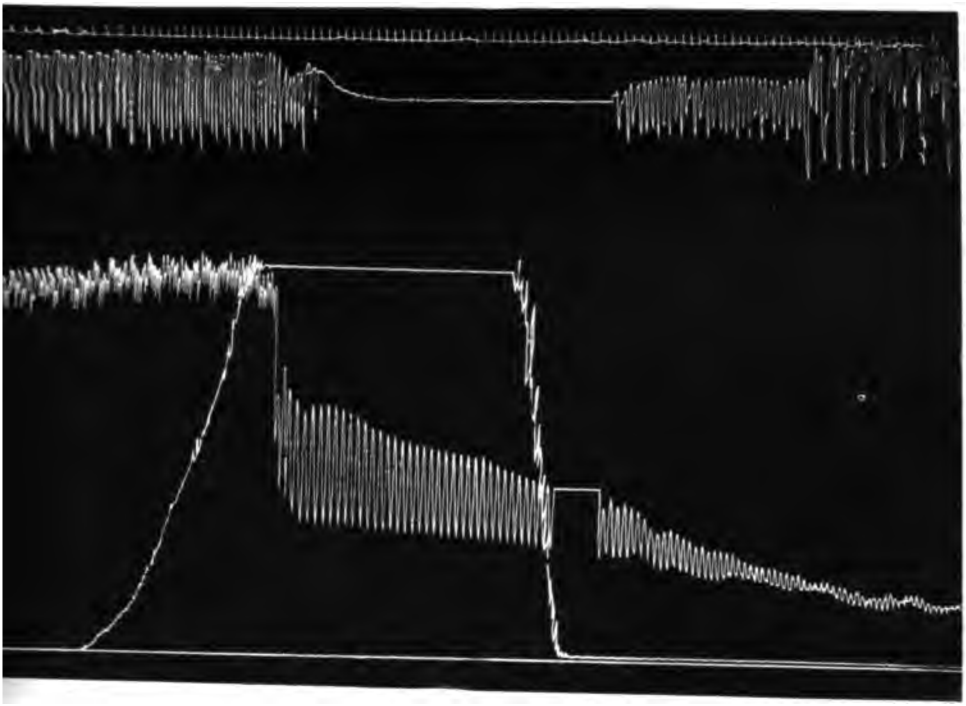

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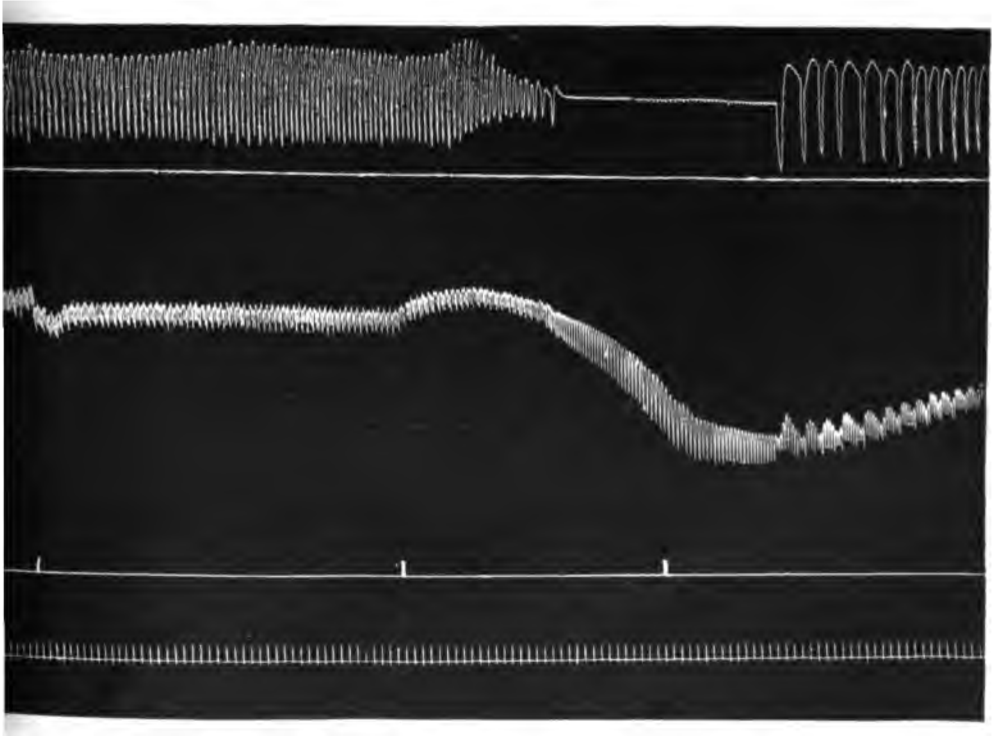

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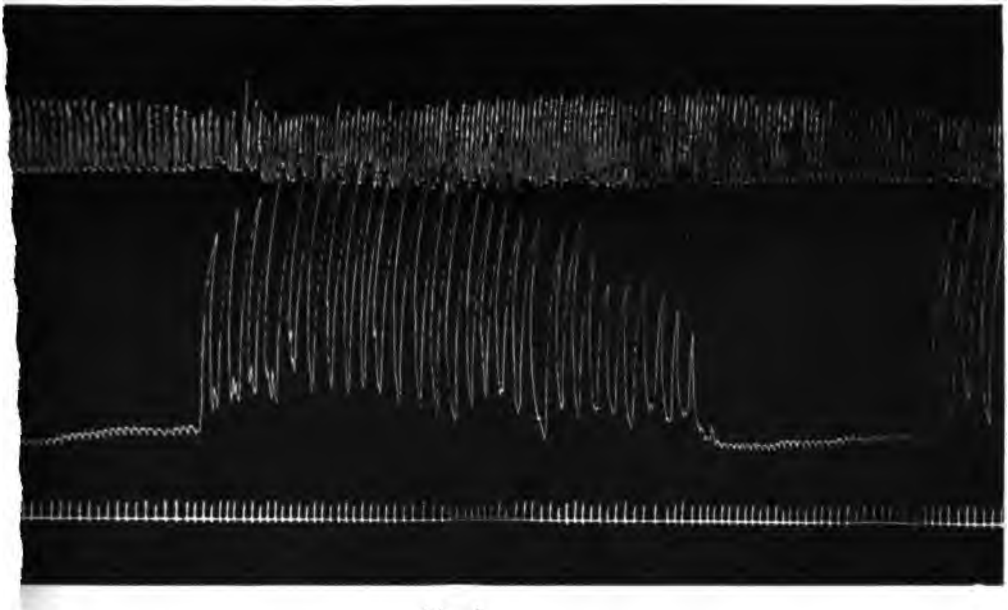

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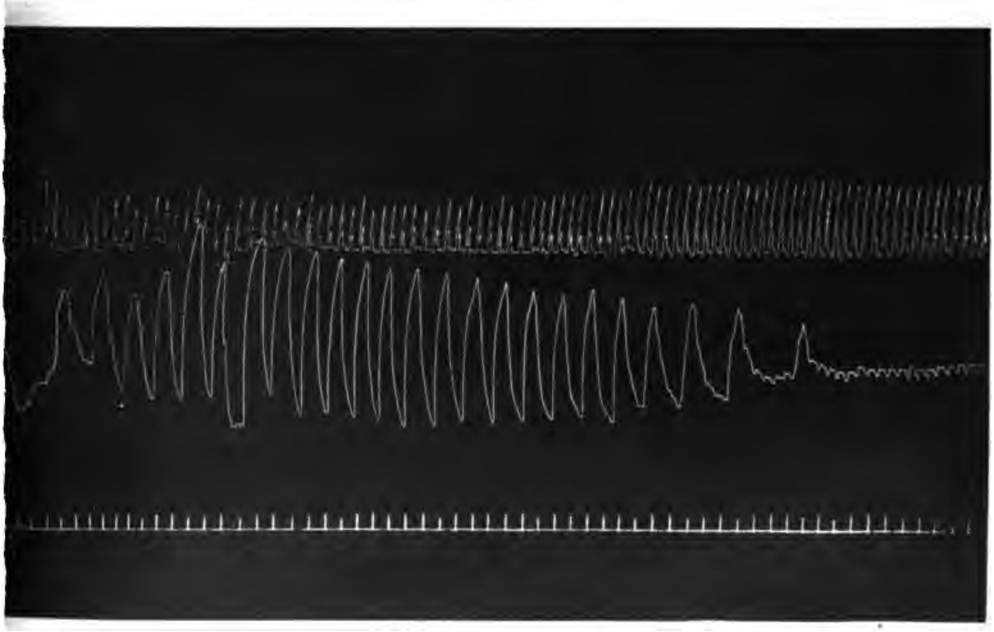
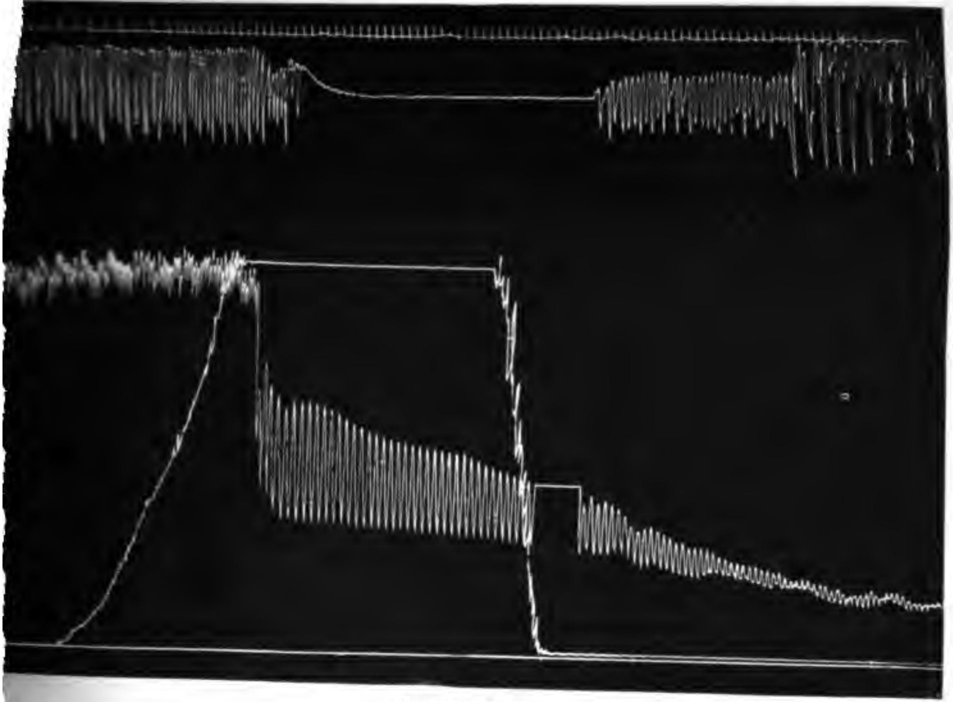


Tracing 22.



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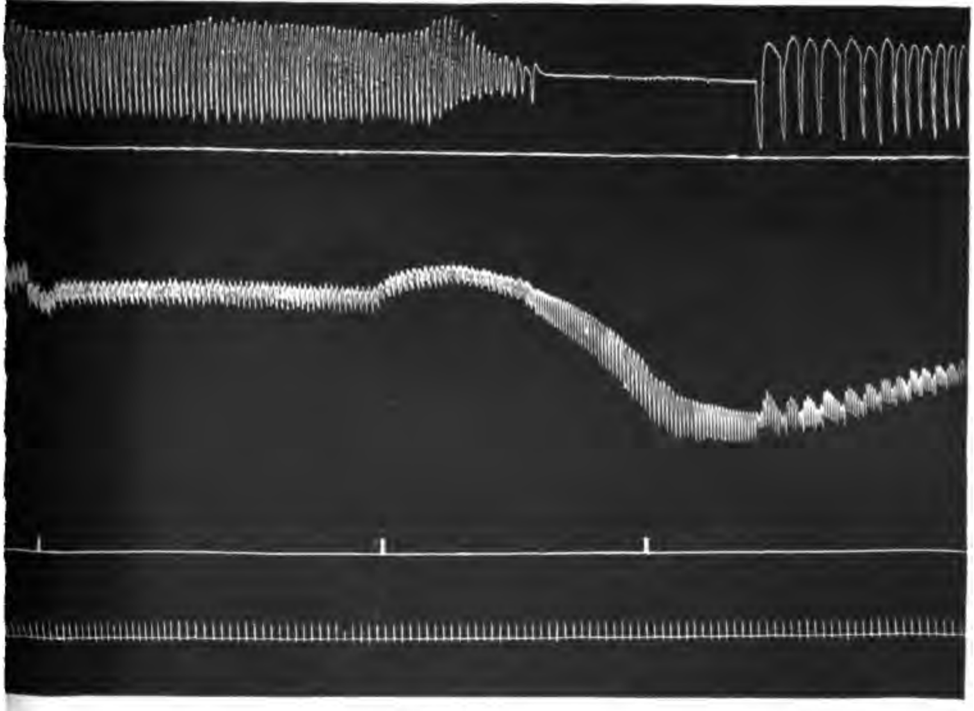

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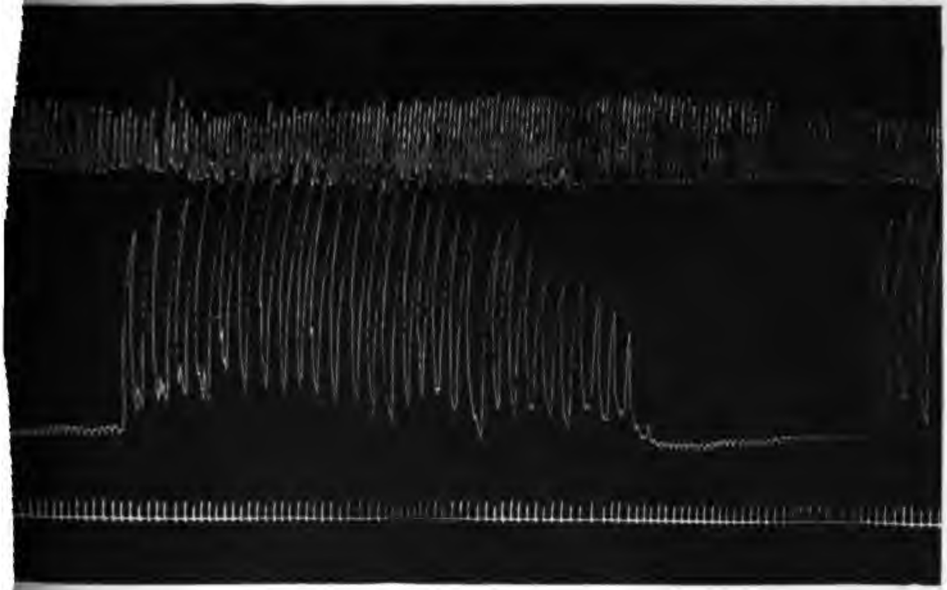

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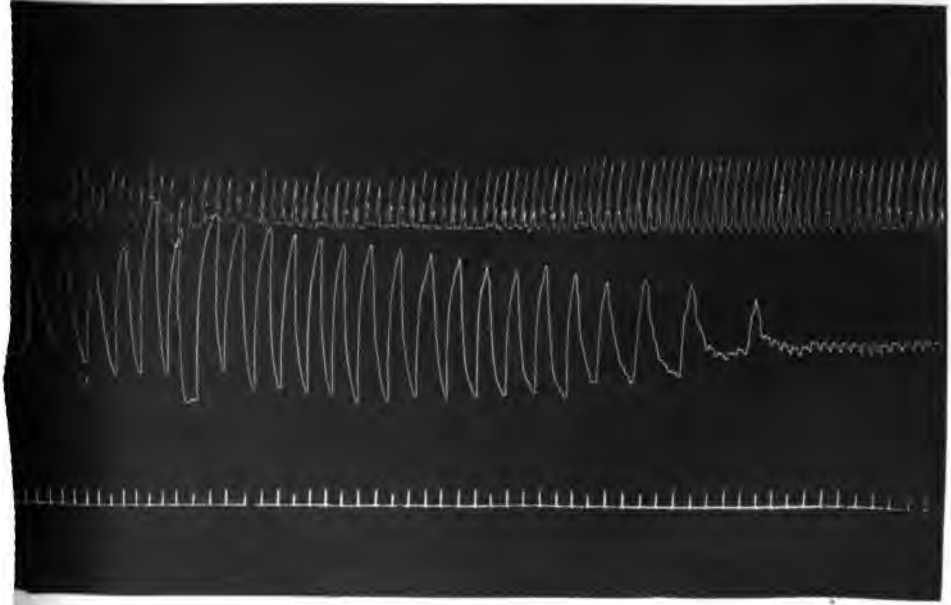

Tracing 22.

racing 20. A single respiration taken during a group of respirations in odic respiration produced experimentally by an increase in intracranial sion. The lowermost line is the base line, the second the intracranial ssure, and the third the blood pressure. The time is in one-second intervals.

racings 21 and 22. Records from clinical cases of periodic respiration occur-
g in association with cardiac and arterial disease. The arrangement of the ves and the interpretation of the records is the same as in Tracings 6-11.

Tracing 23. Similar records taken during the gradual decrease in periodic piration in a patient. Tracing "B" was taken 75 minutes, and "C" 3 hours er "A."

Tracing 24. A similar tracing from a normal subject who breathed period-
lly and endeavored to simulate Cheyne-Stokes respiration.

# THERAPEUTIC EXPERIMENTS WITH ANTICROTAL AND ANTIMOCCASIN SERA.

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The thesis which forms the basis of this paper is the specifi of snake venoms and the therapeutic value of anticrotalus a antimoccasin snake sera. The subject in general is not a n one and has been dealt with in some of the aspects here co-sidered by Calmette,[1] Kanthack,[2] C. J. Martin,[3] Lamb,[4] a Flexner and Noguchi.[5] Calmette's view that cobra antive is active against all kinds of snake venom and even the pois of scorpions has been shown to be erroneous, chiefly by the oth investigators mentioned. The great increase in our knowle of the physiological constitution of venom should make eas explicable the absence of interaction between the antisera of th venom of the Colubridæ and the Viperidæ, but up to the prese time all the tests have been made with Calmette's antivenin, whi is prepared with cobra venom chiefly. My previous studie with Crotalus and Ancistrodon venoms led me to prepare th respective antisera, and to use them in testing first the questio of their specificity, and next the possibility of their employmen herapeutic agents. The wide prevalence of the rattlesnake moccasin in the United States may, at some time, make it rth while to produce commercially antisera for their venoms, e it is proved that they are effective therapeutic agents. experiments were made with anticrotalus and antimoccasin nins made in goats, and anticobra venin kindly supplied by ofessor Calmette. The two main effects investigated were ) the antihæmolytic and (2) the antitoxic.

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* This study was conducted while I was Research Assistant of the Carnegi Institution, Washington, D. C.

[1] Calmette.—Immunisation artificielle des animaux contre le venin des serpents. *La Semaine med.*, 1894, 76.

See also *Ann. de l'Inst. Pasteur*, 1894, vii, 275.

[2] Kanthack.—Rep. of Medical Officer of Loc. Gov. Board, London, 1895–1896, 235.

[3] C. J. Martin.—*Inter-colonial Medical Journal of Australia*, 1897, ii, 537.

Calmette, Ibid., 1898, iii, 192.

[4] Lamb.—Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India, New Series, Nos. 5 (1903), 10 (1904), 16 (1905).

[5] Flexner and Noguchi.—Upon the Production and Properties of Anticrotalus venin. *Journal of Med. Research*, 1904, New Series VI, 363.

In Table I the value of the several antisera in direct and cross utralizing power for several venoms is shown. In A the antitoxic d in B the antihæmolytic values are given. Guinea-pigs were sed for the experiments on toxicity, and defibrinated dog blood, 2.5 per cent. suspension in 0.9 per cent. salt solution, for the ests on hæmolysis. In making the latter tests the blood susensions, venom, and antivenin were kept at 37° C. for two hours nd at room temperature for six hours, after which the readings were made colorimetrically.

The two sets of experiments given show the highly specific antitoxic and antihæmolytic action of the serum. The action is, however, not absolutely specific.

With this knowledge as a basis of further experimentation I proceeded to test the therapeutic value of the antisera. The method was to inject a number of guinea-pigs intraperitoneally with 2 m. l. d. of the venoms and one, two, three, and four hours later to inject the antisera, also intraperitoneally. The control pigs died in from three and a half to four and a half hours after receiving the poison.

Tables II and III give in summarized form the results upon which are based the conclusions that these antisera possess therapeutic properties of high value. Animals which show marked symptoms of venom poisoning can be rescued from certain death provided they are yet able to stand. When they once lie prostrate upon the bottom of the cage they cannot be saved; but I have repeatedly noticed that pigs do not survive this latter condition longer than fifteen to twenty minutes, and hence they are really moribund. In cases in which recovery takes place the improvement proceeds rapidly; the animals are well, apparently, at the end of twenty-four hours, although plete restoration of weight may require several day The successfully treated animals remain well for an i period.

The manner of determining the toxicity of Crotalus is to be learned by referring to a paper by Madsen and N to be published in a subsequent number of this Journal. be stated, however, that one cubic centimeter of the antic serum neutralized *in vitro* 0.0025 gram of the corresponding or 2.5 times the quantity used in the therapeutic experi One cubic centimeter of the antimoccasin serum neutr *in vitro* 0.002 gram of the venom (Table IV).

Calmette[6] and Fraser[7] had already made similar experi with cobra antivenin, and my conclusions are similar to t They state that if a sufficient dose of the antivenin be given w one and a half hours following the injection of a quantity o cobra venom sufficing to cause death in controls in three to hours, the life of the poisoned animals may be saved. As time interval between administrations of venom and antive increased successful treatment became more uncertain. Th experimenters also found that the length of the time inter could in part be overcome by adjusting the dose of the antiven The greater the interval between the poisoning and the an toxin treatment, the larger the quantity of the antivenin requir to rescue the animal, until a point in the poisoning was reache beyond which the antivenin was wholly without influence on th fatal result. This time relation of toxin-antitoxin neutralizatio is met with in diphtheria intoxication (Dönitz[8]) and tetanolysi hæmolysis (Madsen[9]).

## SUMMARY.

1. The action of different antivenins is highly, although not absolutely, specific for the venoms for which they are prepared.

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[6] *Op. cit.*

[7] *Proc. Roy. Soc.*, Edinburgh, 1895, xx, 448.
See also *Brit. Med. Journal*, 1895, Pt. 2, 416.

[8] *Arch. intern. de Pharmacodynamie*, 1899, v, 425.

[9] *Zeitschr. f. Hyg.*, 1899, xxxii, 239.

e antivenomous effect is demonstrable by experiments on icity (*in vivo*) and on hæmolysis (*in vitro*).

2. Anticrotalus and antimoccasin sera possess therapeutic operties of high degree. By their employment before the stage extreme prostration has been reached, poisoned guinea-pigs n be saved.

3. The standardization of antivenins must be made separately r the antitoxic and antihæmolytic actions, since these do not ear a constant and invariable relation to each other.

## TABLE I.

### A.—CROSS ANTITOXIC ACTION.

| | Protected against | | |
|---|---|---|---|
| | Crotalus venom | Water moccasin venom | Cobra venom |
| Anticrotalus 2.5 c.c. | 12 m.l.d. | 1 m.l.d. | 0 |
| Antimoccasin 2.5 c.c. | 3 m.l.d. | 5 m.l.d. | 0 |
| Anticobra 2 c.c. | 0 | 1 m.l.d. | 5 m.l.d. |

0 = no protection.

### B.—CROSS ANTIHÆMOLYTIC ACTION.

| | Protected against | | | | |
|---|---|---|---|---|---|
| | Crotalus | Cobra | Moccasin | Daboia | Trimeresurus |
| Anticrotalus 1 c.c. | 10 m.h.d.* | 0.75 m.h.d. | 1.33 m.h.d. | 1.5 m.h.d. | 2.25 m.h.d. |
| Antimoccasin 1 c.c. | 4.125 m.h.d. | 4 m.h.d. | 40 m.h.d. | 4 m.h.d. | 5 m.h.d. |
| Anticobra 1 c.c. | 0 | 2 m.h.d. | 0 | 0 | 0.3 m.h.d. |

* M.h.d. = minimal complete hæmolytic dose for 8 c.c. of 2.5% suspension of defibrinated dog blood in 0.9% saline solution read after 2 hours' incubation at 37° C.

0 = no protection.

## TABLE II.

Therapeutic experiments with anticrotalus serum.

Unfiltered Crotalus venom and anticrotalus goat serum filtered through a Chamberland bougie.

Test animals: guinea-pigs of 280 grams; injected intraperitoneally. Apr 20, 1904.

| Venom in grams | Symptoms before the injection of antivenin | Antivenin | | Symptoms after the injection of antivenin | Result |
|---|---|---|---|---|---|
| | | Time after injection of venom | Dose | | |
| 0.001 | Immediate irritating effect.<br>20 minutes: Abdominal tension steadily increasing; unable to walk. Gentle handling causes pain and cries; hair ruffled.<br>30–60 minutes: High degree of abdominal tension; wall presents dark purplish color. No resistance to handling, but animal still able to stand<br>1½ to 2 hours: Symptoms described increase gradually.<br>3 hours: Animal down; labored respirations; subnormal temperature. Died. | | 0 | | † 3 h. 48 m. |
| 0.001 | Symptoms similar to preceding but collapsed more slowly; able to stand until 4 hours 17 minutes after injection. | | 0 | | † 4 h. 34 m. |
| 0.001 | 50 minutes: Abdominal tension high; wall discolored by extravasation of blood; still able to stand, but unable to run. | 1 hour | 1 c.c. | 15 minutes: Very sick. 30 minutes: Condition unchanged, but later no increase in symptoms. A hæmo-serous fluid escaped from the needle hole.<br>1 hour: No change.<br>2 hours: Condition steadily improving.<br>4 hours: Able to run, but abdominal tension still remains high.<br>7 hours: Somewhat sick.<br>12 hours: Eats and runs about; local discharge drying up.<br>24 hours: Normal except for slight abdominal tension; weight 270 grams.<br>2 days: Well, weight 280 grams. | § |

| Venom in grams. | Symptoms before the injection of antivenin | Antivenin. | | Symptoms after the injection of antivenin. | Result. |
|---|---|---|---|---|---|
| | | Time after injection of venom. | Dose. | | |
| 0.001 | 1 hour and 50 minutes. Symptoms grave, scarcely able to stand; heart weak and quick. | 2 hours | 1 c.c. | 15 minutes: Condition unchanged.<br>4 hours: Critically ill, and fell. Died 4 hours and 30 minutes after the injection of antivenin. | + 6 h. 30 m. (or 4 h. 30 m. after the injection of antivenin.) |
| 0.001 | 1 hour and 50 minutes. Symptoms similar to preceding. | 2 hours | 2 c.c. | 1 hour and 30 minutes: Condition unchanged.<br>2 to 3 hours: Steady improvement.<br>18 hours: No more general symptoms; abdominal tension reduced, animal runs.<br>2 days: Complete recovery, weight 290 grams. | § |
| 0.001 | 2 hours and 50 minutes: Unable to move; critically ill. | 3 hours | 1 c.c. | 40 minutes: Condition unchanged.<br>80 minutes: Bloody fluid escaped from the needle puncture. Died 2 hours and 5 minutes after injection. | + 5 h. 5 m (or 2 h. 5 m. after the injection of antivenin.) |
| 0.001 | 2 hours and 50 minutes: Symptoms similar to preceding. | 3 hours | 4 c.c. | 2–3 hours: Condition slowly improved.<br>24 hours: Hæmo-serous fluid escaped from the needle hole. Abdominal tension still somewhat high; wall is of dark purple color. Able to run and eat. Weight 250 grams.<br>2 days: No general symptoms; local condition improving.<br>4 days: Well; weight 270 grams. | § |
| 0001 | 3 hours and 50 minutes: Symptoms similar to preceding. | 4 hours | 4 c.c. | 30 minutes: Seriously ill.<br>2 to 3 hours: No change. Died. | + 8 h. 45 m (or 4 h. and 45 m. after injection of antivenin.) |
| 0.001 | Symptoms similar to preceding. | 4 hours | 8 c.c. | General improvement after 8 hours.<br>Next morning well.<br>Recovery in 3 days; no complication after many months. | § |

## TABLE III.

Therapeutic experiments with antimoccasin serum.
Water moccasin venom and filtered antiserum from goat.
Guinea-pigs of 420 grams; intraperitoneal injection, June 9, 1904.

| Venom in grams. | Symptoms before the injection of antivenin. | Antivenin. | | Symptoms after the injection of antivenin. | Result. |
|---|---|---|---|---|---|
| | | Time after injection of venom. | Dose. | | |
| 0.0024 | Immediate irritating effect.<br>15 to 20 minutes: Abdominal tension increasing steadily.<br>30 to 45 minutes: Hair erect; dyspnœic; unable to run.<br>1 hour: Marked dyspnœa and weakness; still slight resistance to handling.<br>2 hours: Unchanged.<br>3 hours: Unable to move; marked dyspnœa; paresis.<br>4 hours: Quick and shallow respiration; lies on side after 4 hours and 25 minutes.<br>Died 4 hours and 40 minutes after the injection. | | 0 | | † 4 h. 40 m. |
| 0.0024 | Similar symptoms to preceding. Died. | | 0 | | † 4 h. 35 m. |
| 0.0024 | Similar symptoms to preceding, died of dyspnœa and paralysis. | | 0 | | † 4 h. 20 m. |
| 0.0024 | Similar symptoms to preceding, but quicker collapse. | | 0 | | † 3 h. 50 m. |
| 0.0024 | Very weak just before injection; still able to move; high abdominal tension. | 1 hour | 1 c.c. | Animal gradually worse; down after 9 hours. Dyspnœa. | † 10 h. |
| 0.0024 | Similar symptoms to preceding. | 1 hour | 2 c.c. | Improvement commenced after 3 hours and became more rapid after 10 hours.<br>Next morning animal well, eats and runs.<br>No loss of weight. No complication followed the experiment. | § |
| 0.0024 | Marked dyspnœa, paresis, unable to run. | 2 hours | 2 c.c. | General symptoms became steadily more serious. Died. | † 3 h. 35 m. |

| Venom in grams. | Symptoms before the injection of antivenin. | Antivenin. | | Symptons after the injection of antivenin. | Result. |
|---|---|---|---|---|---|
| | | Time after injection of venom. | Dose. | | |
| 0.0024 | Similar condition to preceding. | 2 hours | 3 c.c. | No improvement; died of respiratory paralysis. | † 6 h. 20 m. |
| 0.0024 | Similar condition to preceding. | 2 hours | 4 c.c. | Improvement after 2 hours and animal runs after 6 hours. Next morning animal well Regained weight in 5 days. | § |
| 0.0024 | About same as above, but more dyspnœic. | 2 hours | 6 c.c. | Improvement after 2 hours; much better in 5 hours. 10 hours later animal runs. Next morning animal well. Weight regained in 4 days. | § |
| 0.0024 | Dyspnœic, atonic, unable to walk, abdominal tension high. | 3 hours | 4 c.c. | No improvement. | † 4 h. 10 m. |
| 0.0024 | Same condition as above. | 3 hours | 8 c.c. | Improvement started after 10 hours; animal weak after 24 hours. Regained weight in 2 days. No complications in many months. | § |
| 0.0024 | Marked dyspnœa and abdominal tension, unable to move. Critically ill. | 4 hours | 10 c.c. | Much improved after 10 hours. Next morning, well. No loss of weight. | § |

## TABLE IV.

Summary of therapeutic experiments.

The number of minimal lethal doses employed was 2, namely, 0.001 gram of Crotalus adamanteus and 0.0024 gram of water moccasin venom.

| Anticrotalus serum. | | | Antimoccasin serum. | | |
|---|---|---|---|---|---|
| Time. | Dose. | Result. | Time. | Dose. | Result. |
| Control | 0 | † 3 h. 48 m. | Control | 0 | † 4 h. 40 m. |
| | 0 | † 4 h. 34 m. | | 0 | † 4 h. 35 m. |
| | 0 | † 4 h. 20 m. | | 0 | † 4 h. 20 m. |
| | 0 | † 4 h. 50 m. | | 0 | † 3 h. 50 m. |
| 1 hour | 1 c.c. | § | 1 hour | 1 c.c. | † 10 h. |
| | | | | 2 c.c. | § |
| 2 hours | 1 c.c. | † 6 h. | 2 hours | 2 c.c. | † 3 h. 35 m. |
| | 2 c.c. | § | | 3 c.c. | † 6 h. 20 m. |
| | | | | 4 c.c. | § |
| 3 hours | 1 c.c. | † 5 h. | | 6 c.c. | § |
| | 4 c.c. | § | 3 hours | 4 c.c. | † 4 h. 10 m |
| | | | | 8 c.c. | § |
| 4 hours | 4 c.c. | † 9 h. | | | |
| | 8 c.c. | § | 4 hours | 10 c.c. | § |

† = Death.
§ = Recovery.

## ADDENDUM.

A brief account of the foregoing experiments was re the British Medical Association, at Oxford, July 28, found, on my return to America, that Dr. George L turned his attention to this subject of the specificity venin. Dr. Lamb employed several kinds of venom cephalus, Daboia, etc.) and cobra and daboia antivenins concluded that their actions are highly specific. In Mar Dr. Flexner received from Dr. Lamb several small vials daboia serum together with a liberal sample of the venom, t which I was given the opportunity to test their reciprocal against Crotalus venom and antivenin. The accompanyin tocols show that the action of each of these venoms and anti is highly if not absolutely specific.

Guinea-pigs of 220 to 250 grams were injected intraperiton with venom or antivenin-venom mixtures. The venom solu were freshly made, and the length of contact of venom and a venin before injection was two hours at 37° C.

The toxicity of daboia venom was found to be as follows: d of 0.0006 to 0.0001 gram killed guinea-pigs in about six hou 0.00005 gram in nine hours; 0.000045 gram caused much l of weight, but was not fatal; 0.00002 gram produced no sympton Hence 0.00005 gram of daboia venom contained one m.l.d. f guinea-pigs of 200 grams.

In neutralizing with the antidaboia serum the following result were obtained: 0.0004 gram daboia venom (= 8 m.l.d.) was completely neutralized by 0.5. c.c. of the antivenin; 0.2 c.c. of antivenin was not sufficient to neturalize the venom. The mixture of 0.1 c.c. of the serum with 0.0004 gram of venom was insufficient to reduce its toxicity to a subminimal lethal dose, but contained less than two m.l.d., as half of the mixture caused only a slight intoxication.

In another series of experiments 0.5 c.c. of antidaboia serum was added to 0.002 gram (40 m.l.d.) of the venom, after which the number of m.l.d. still present in the mixture was determined. It was found that the whole mixture contained a dose which produced death in twelve hours; half of the mixture, in wenty hours; one third caused no symptoms. Therefore it can e stated that 0.5. c.c. of antidaboia serum reduced the number f m.l.d. from forty to between two and three.

The action of antidaboia serum upon Crotalus venom was tudied to determine its specificity. Intraperitoneal injections of 0.0005 gram Crotalus venom killed guinea-pigs of 200 grams weight in seventeen hours; 0.00045 gram was not fatal.

To 1 c.c. of antidaboia serum, capable of neutralizing nearly 75 m.l.d. of daboia venom, were added amounts of Crotalus venom representing one, two, three, four, five, six, and eight m.l.d. The mixtures after two hours' contact were injected into the guinea-pigs. The results may be summarized as follows:

| | | | | | | |
|---|---|---|---|---|---|---|
| Antidaboia | serum | 1 c.c. | Crotalus | venom | 0.0005 (1 m.l.d.) | Survived |
| " | " | " | " | " | 0.001 (2 m.l.d.) | " |
| " | " | " | " | " | 0.0015 (3 m.l.d.) | " |
| " | " | " | " | " | 0.002 (4 m.l.d.) | Died in 40 hours |
| " | " | " | " | " | 0.0025 (5 m.l.d.) | " " 9 " |
| " | " | " | " | " | 0.003 (6 m.l.d.) | " " 12 " |
| " | " | " | " | " | 0.004 (8 m.l.d.) | " " 12 " |

Autopsies upon the dead animals showed marked hæmorrhage in thorax, abdomen, and diaphragm. Hence 1 c.c. of antidaboia serum neutralized only about 3 m.l.d. of Crotalus venom, thus showing its highly specific nature.

For testing the antihæmolytic action of antidaboia serum 3 per cent. defibrinated dog blood, in 0.9 per cent. NaCl solution, was employed. Into each tube 8 c.c of the suspension were measured, to which the desired quantity of venom solution was added. The venom-antivenin mixture was kept for two hours at 37° C. before testing. Before reading the results the mixtures were kept at a temperature of 37° C. for two hours and at 20° C. for four hours.

The complete hæmolytic dose of daboia venom was found to be 0.0035 c.c. of 0.4 per cent. daboia venom solution per 8 c.c. of 3 per cent. defibrinated dog blood suspension. The m.h.d. of Crotalus venom was 0.015 c.c. of a 0.4 per cent. solution under the same conditions. It was found further that 1 c.c. of this daboia antivenin neutralized completely 0.1 c.c. 0.4 per cent. daboia venom solution, and only 0.035 c.c. of 0.4. per cent.

Crotalus venom solution. Hence the antihæmolytic
the antivenin is highly specific.

Antidaboia serum 1 c.c. neutralized daboia venom 28.57 m.h.
" " " " Crotalus " 2.3 "

This serum also showed feeble antihæmolytic powe
moccasin venom, but no power against cobra venom.

# EXPERIMENTAL ANÆMIAS IN THE RABBIT.*

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The experimental and anatomical studies which form the basis of this paper were begun under the direction of Dr. Flexner in the Pathological Department of the University of Pennsylvania, and have been continued under Professor Welch in the Pathological Laboratory of the Johns Hopkins University. They have had as their object primarily the demonstration of the structure of the bone marrow and its reaction to various stimuli, while, at the same time, it was hoped that the knowledge thus gained might throw light on some of the pathological processes affecting the hæmopoietic system.

In the earlier experiments immune sera obtained by immunizing geese to the various blood-producing organs and the blood of the rabbit were used and the results of these experiments were published at that time.[1] In subsequent experiments I have employed various substances, chiefly, however, ricin and saponin, which have a marked effect on the blood and blood-forming organs and which can be more easily standarized as to dose and are more easily obtained than the artificial sera. Before entering upon a discussion of these experiments, I shall quote some of the conclusions published as a result of my early work, as they to a certain extent form a thesis for the later work. From a study of the blood and of the tissues of the hæmopoietic organs of the animals used, the following conclusions were drawn:

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* This paper was read before the American Association of Pathologists and Bacteriologists, May 19, 1906.

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[1] Bunting, The Effects of Lymphotoxins and Myelotoxins on the Leucocytes of the Blood and on the Blood-Forming Organs, *Univ. of Penn. Med. Bulletin*, 1903, xvi, 200.

"Leucocytosis is the excessive reaction of the leucoblastic tissues to a leucopenia of the circulating blood. This leucopenia may be due to the withdrawal of leucocytes from the circulation or to their destruction within the circulation. The amphophile, eosinophile, and basophile leucocytes are derived from the marrow. The lymphoid cells are chiefly derived from the lymph-glands and the spleen. The marrow however is a lymphoid tissue and contains typical lymphocytes. The lymphocyte is amœboid. Amphophile and eosinophile myelocytes may multiply by mitosis. Their number may also be increased by the development of specific granules in the protoplasm of large mononuclear elements with scant basophilic protoplasm, the least differentiated cell of the marrow and identical in appearance with the cells of the germinal centres of the lymph-glands. Basophilic cells are formed by the development of basophilic granules in mononuclear cells. Multiplication by mitosis is not excluded by negative findings. Megaloblasts are a constituent of normal marrow and form the proliferating centre of erythroblastic tissue."

In brief as a result of this study the conception was obtained that the red marrow of the rabbit normally consists essentially of a patch-work of cells arranged in groups or proliferating centres; certain groups composed of indifferent myeloblasts at the centre surrounded by myelocytes and on the periphery close to the capillaries the mature polymorphonuclear cells; others composed of immature red cells, or even indifferent myeloblasts at the centre and surrounding layers of megaloblasts, intermediate red cells, and normoblasts and peripheral layers of mature red cells; while still others show lymphoid cells forming the peripheral layers. I emphasize this conception of the structure of the marrow because it is of great importance in understanding the reactions of the marrow and seems definitely to be demonstrated by these experimental and anatomical studies. While such centres may not always be demonstrable in sections of an actively proliferating and hence crowded marrow, if one keeps in mind the fact of the necessity of the plane of section passing through the centre of the groups in order to demonstrate the true relation of the cells, he will find a sufficient number of ch groups to convince him it is the rule of structure. Due lowance must be made for amœboid motion of certain cells, r numerous large mononuclear cells apparently inactive and ot forming a part of any definite group, and for the changing elation of fat cells. In many cases these groups do not form definite spherical bodies, but rather a sheet or layer of cells backed by a fat cell with the immature blood cells lying nearest the fat cells, and the mature forms on the periphery. This arrangement and grouping of cells in the marrow are best seen when the marrow has been depleted of certain of its mature cells, as for example of polymorphonuclear cells after an intraperitoneal or intrapleural injection of aleuronat. This is well shown by the marrow of Rabbit A. R. C.

EXPERIMENT I.—Rabbit A. R. C., Belgian hare.

ii—19—'06. R. b. c. 5,800,000, w. b. c. 6,250.

ii—21—'06. 10 A.M., intraperitoneal injection of 7 c.c. of sterile aleuronat suspension.

ii—22—'06. 10 A.M., w. b. c. 9,250. Animal killed.

In the peritoneal cavity there is found a considerable exudate of an opaque white, moist, sticky nature adherent to the peritoneal surface of the intestine. The mesenteric lymph glands are slightly swollen, moist on section, and have prominent follicles. The bone marrow of the femur is light red in color, slightly translucent, and increased in consistency. Other organs are negative. Microscopical examination of the exudate shows it to be composed almost entirely of polymorphonuclear leucocytes.

On examining the marrow of this rabbit, it was found to be relatively poor in cells. The capillary vessels stood out sharply, the fat cells were shrunken and filled with a granular eosin-staining precipitate. Scattered between the fat cells were numerous small groups of mononuclear cells, myelocytes, usually with not more than four or six cells to a group. Scarcely a polymorphonuclear cell was to be found in the whole marrow. The erythrogenetic groups however were larger and were apparently little disturbed by the sterile inflammation, groups of megaloblasts and their peripheral normoblastic cell rows being conspicuous by reason of their intensely staining nuclei and the depletion of the surrounding part of the marrow of cells. A few mitotic figures were present in myelocytes.

In this marrow then one sees the result of the transfer of the mature polymorphonuclear cells to the field of inflammation. They have all been withdrawn and the myelocytic centres of the groups left. Regeneration of cells has not taken place to any extent, or if it has, the cells have been withdrawn as rapidly as they have been formed. Scattered mitoses show that the process was beginning.

An entirely different picture is afforded by the marrow of animals killed at a later date as shown by Rabbit A. R. B.

Experiment II.—Rabbit A. R. B., Belgian hare.
ii—7—'06. 10 A.M., r. b. c. 6,080,000, w. b. c. 8,800.
ii—7—'06. 2 P.M., intraperitoneal injection of 5.5 c.c. of sterile aleuronat suspension.
ii—8—'06. 9 A.M., w. b. c. 11,250.
ii—9—'06. 11.30 A.M., w. b. c. 12,500. Animal killed.

The intestines are found glued together by a sticky, opaque white exudate. Mesenteric lymph glands are slightly enlarged, and quite moist. Bone marrow of the femur is of a grayish pink color, rather opaque and of moderate firmness. Other organs are normal. The exudate shows microscopically a polymorphonuclear cell content.

On examination of this marrow it was found extremely cellular. Large groups of myelocytes were present with numerous mitotic figures and on the periphery of these groups were numerous polymorphonuclear leucocytes. The erythrogenetic centres were less prominent than in the marrow just described by reason of the great increase in the leucogenetic centres, which were exaggerated above the normal, the reaction being in excess. The fat cells were small, and appeared almost crowded out by the cells of the blood group.

Similar marrow pictures were obtained in rabbits which had received intravenous injections of staphylococcus aureus cultures, with the exception that fragmented nuclei in the marrow gave evidence of slight direct injury.

I shall refer to this arrangement of cells in the marrow throughout this paper in explanation of the results following the application of various stimuli to the hæmopoietic system. In studying the reactions of the marrow it seems essential to follow the progress of the experiments by frequent and careful blood counts combined with a study of the stained blood smears, and that has been done in this series of experiments. The blood has been counted according to the accepted clinical technique with the Thoma-Zeiss apparatus and all the counts have been made by the author, to rule out the personal equation as a factor of error. The smears have been stained by the Jenner stain, which has been found very satisfactory for rabbit blood in that it gives a sharp differentiation of the cell nuclei and allows of easy determination of the basophilic cells which form a relatively large percentage of the leucocytes. In making differential counts 500 cells have been counted, and the number of nucleated red cells per cubic millimetre has been determined from the number seen in counting 500 leucocytes, although it has been found possible to count the nucleated red cells directly in the acetic acid solution, while counting the leucocytes, owing to the higher refraction of the nuclei of the red cells together with a slight variation in color from the greenish color of the white cell nuclei. These counts have been found to agree closely with those obtained by the smear method when any considerable number of nucleated red cells is present.

There has been some criticism of the use of the rabbit as an experimental animal in blood work on the ground that the number of leucocytes in the peripheral circulation was subject to marked variation with the slightest change in the conditions of the animal, even those incident to handling the animal while making the count. Such has not been my experience. Counts made on successive days have shown only the slightest variation, either in the total count or in the differential. It has been found with ricin that for a fixed dose one may almost predict the blood count at a stated time following, with such machine-like regularity does the animal react. I have found it most satisfactory to take the blood from the marginal vein, waiting after the puncture until a free flow is established. Repeated counts may be made from the vein without its becoming thrombosed.

The action of ricin on the blood and marrow has been followed by this method of study over quite a series of cases. This toxin was first employed at the suggestion of Dr. Flexner, who had noted in sections from animals treated with ricin numerous nucleated red cells in the capillaries and subperitoneal hæmorrhages. Ricin has a marked toxic action on all the blood elements. The action on the leucocytes is shown in stained blood smears, in which the polymorphonuclear cells show both pyknosis and karyolysis and not infrequently complete fragmentation of the cell, small fragments of amount of protoplasm containing the specific frequent finding. The mononuclear cells shov olysis though pyknosis also occurs. Of the red in the animal body hæmolysis as is shown by tl count, frequent hæmoglobinuria, and accumulat in the spleen pulp and lymph-glands.

The specimen of ricin I employed was fatal to *tl* 36 hours in a dose of from $\frac{1}{40}$ to $\frac{1}{20}$ milligram *pe* body weight. The larger doses prove fatal as a hours. During this time the effect on the leucocyt venous dose is the most noticeable. A sharp *dimi* number of red cells is not always *immediately a* probably to the abstraction of body fluids *during tl* which is caused by the toxin. The *immediate ef* leucocytes of a fatal dose of the poison is a *destructi* the resulting leucopenia proves on analysis *to be ca* diminution in both leucocytes and lymphocytes. *W* dose this leucopenia may not be recovered *from in* although the minimum count is reached before *that ti* reactionary increase in the polymorphonuclear cells *ta* usually though not always somewhat earlier *than tha* lymphocytes. The effect on the leucocytes of a dose *fata* 24 hours is shown by the protocol of Rabbit *R. R. H.*

EXPERIMENT III.—Rabbit R. R. H., albino, weight 1540 grms.
ii—11—'03. 11 A.M., w. b. c. 7,000.
12 NOON, intravenous injection of $\frac{1}{10}$ mg. of *ricin, in* salt solution.

2 P.M., w. b. c. 4,800.
6 P.M., w. b. c. 9,300.
8 P.M., w. b. c. 9,000.
ii—12. 8.30 A.M., animal died. Postmortem examination shows *subcutane* mesenteric, and intestinal lymphoid structures mo ately swollen, with minute hæmorrhages. There slight hæmorrhages into the omentum. Spleen apparen not enlarged. Liver free from lesions. Bone marro firm, dark red, and showing areas of hæmorrhag Thymus large and containing punctate hæmorrhages Bladder is filled with dark reddish brown urine. Other organs normal.

Differential count of leucocytes, February 11:

| | 11 A.M. | 2 P.M. | 6 P.M. | 8 P.M. |
|---|---|---|---|---|
| Pseudoeosinophiles | 3,220 | 1,410 | 8,184 | 6,930 |
| Basophiles | 126 | 154 | 37 | 108 |
| Eosinophiles | 56 | 39 | 56 | 36 |
| Small mononuclears | 3,066 | 2,977 | 800 | 1,638 |
| Large mononuclears | 532 | 220 | 223 | 288 |
| Total | 7,000 | 4,800 | 9,300 | 9,000 |

In animals which survive longer than 24 hours there is a sharp reactive leucocytosis in which the polymorphonuclear elements take the larger part. This will be brought out in protocols given later on. This leucocytosis varies somewhat, for example, Rabbit R. R. C. before injection showed 8,750 leucocytes, 24 hours after 16,750; R. R. D. 12,000 before, 36,875, 24 hours after; R. R. O. 6,250 before and 15,750, 24 hours after; R. R. R. 15,000 before and 40,000 at the end of 48 hours. In the stained smears however the leucocytes are found to show even in the period of reaction to a great extent the signs of toxic injury in karyolysis and in fragmentation of the nucleus. In extreme cases scarcely a normal cell may be found.

To summarize briefly, then, the effect on the leucocytes of a fatal dose of ricin is a destructive one leading to a leucopenia of a few hours' duration followed by a sharp reaction which reaches a considerable height at the end of 24 hours, but among the cells of this leucocytosis are many that show signs of injury. An explanation of this last point is to be looked for in the condition of the marrow which will be described in connection with the red-cell changes.

Although, as has been noted, immediately after a fatal dose of ricin there is little change in the count of red cells, by far the most interesting phase of the activity of the toxin is its effect on them, and this is shown by the early appearance of nucleated red cells in the circulation. Nucleated red cells are not found in the circulation of a perfectly healthy rabbit. In animals confined to laboratory basements without adequate sunlight, etc., a single normoblast may be found here or there in a smear.

After ricin, administered intravenously, nucleated red cells appear in the circulation within a few hours, increasing in number rapidly during the first 24 hours, their number depending apparently on two factors, the dose of toxin and the state of the erythrogenetic tissue of the marrow. The nucleated forms consist of normoblasts, in greatest numbers, pyknotic normoblastic nuclei without a surrounding rim of protoplasm, and megaloblasts in small numbers, understanding by a megaloblast a large cell with hæmoglobin-containing protoplasm and a reticular nucleus equal to or exceeding the size of a mature red blood cell. The early appearance of the nucleated red cells is shown by the following experiments.

EXPERIMENT IV.—Rabbit R. R. E., gray rabbit.

xii—10—'02. 12 NOON, w. b. c. 6,400.
2 P.M., ½ mg. ricin injected in ear vein.
5 P.M., w. b. c. 4,800.
xii—11—'02. Animal died during night with typical postmortem findings of ricin poisoning.

Analysis of blood count:

| | 12 NOON | 5 P.M. |
|---|---|---|
| Polymorphonuclears | 3,187 | 3,485 |
| Small mononuclears | 2,855 | 931 |
| Large mononuclears | 358 | 384 |
| Total | 6,400 | 4,800 |
| Normoblasts | | 19 |

EXPERIMENT V.—Rabbit R. R. F., gray rabbit.

xii—18—'02. 10.03 A.M., given ½ mg. ricin intravenously. Blood counts made before administration of dose and at intervals of 2, 4, 6, and 8 hours afterward. The analyzed count is as follows:

| | Before | 2 hrs. Afterward | 4 hrs. Afterward | 6 hrs. Afterward | 8 hrs. Afterward |
|---|---|---|---|---|---|
| Polymorphonuclears | 4,098 | 4,214 | 1,294 | 537 | 1,800 |
| Small mononuclears | 2,516 | 2,478 | 659 | 455 | 2,412 |
| Large mononuclears | 261 | 308 | 147 | 108 | 288 |
| Total | 6,875 | 7,000 | 2,100 | 1,100 | 4,500 |
| Normoblasts | — | — | — | 50 | 504 |
| Megaloblasts | — | — | — | 14 | 54 |
| Naked nuclei | — | — | — | 6 | 153 |

In these experiments the red counts were not made and the dosage was so great that the animals did not survive for 24 hours.

In animals which survive to that period the nucleated red-cell reaction is sharp, large numbers being present in the circulation. This is well shown by the count of Rabbit R. R. I.

EXPERIMENT VI.—Rabbit R. R. I., mottled rabbit, 1,800 gms. in weight.
x—24—'04. 10 A.M., r. b. c. 5,364,000, w. b. c. 10,500.
10 A.M., $\frac{1}{10}$ mg. ricin in salt solution injected in ear vein.
4 P.M., r. b. c. 5,208,000, w. b. c. 7,750, no nucleated red cells.
x—25—'04. 10 A.M., r. b. c. 5,464,000, w. b. c. 16,250, normoblasts 1,072, megaloblasts 162, naked red-cell nuclei 910 per cu. mm. (determined from differential count which is here omitted).
x—25—'04. 11 P.M., animal found dead. Postmortem examination shows all lymphatic structures swollen, injected, and containing minute hæmorrhages. Spleen is large, soft, dark purplish in color. Liver shows many scattered opaque yellowish dots, evidently foci of necrosis. No omental or mesenteric hæmorrhages. Bone marrow dark red, firm, and showing minute hæmorrhages. Other organs normal except for some opacity of tissues. Tissues hardened in Zenker's fluid.

A similar blood picture was given by Rabbit R. R. C. 24 hours after a dose of $\frac{1}{8}$ milligram of ricin, when with a leucocyte count of 16,750, there were present 1,909 nucleated red cells per cu. mm. in the circulation. Rabbit R. R. O. with red cells 4,596,000, leucocytes 6,250, and with no nucleated red cells before the administration of the toxin, gave 24 hours after $\frac{1}{40}$ mg. of ricin a red count of 4,520,000 cells, a leucocyte count of 15,750, and showed 620 normoblasts and 124 naked red-cell nuclei per cu. mm. but no megaloblasts.

That the intensity of this striking nucleated red-cell reaction depends largely on the condition of the marrow seems to be definitely shown by two subsequent experiments. The first of these was made on a rabbit, which though showing a normal red-cell count before the ricin was given, showed on the differential count of the stained preparations a considerable number of normoblasts, evidence, apparently, that the bone marrow was being pushed to maintain the red count at the normal figure. The only pathological finding which might explain this condition was the presence in the peritoneal cavity of numerous cysticerci.

Experiment VII.—Rabbit R. R. R., weight 1,430 gms.
iii—30—'05. r. b. c. 5,480,000, w. b. c. 15,000, normoblasts 90.
2 P.M., given $\frac{1}{30}$ mg. ricin intravenously.
iv—1—'05. 2 P.M., r. b. c. 5,488,000. w. b. c. 40,000, normoblasts 12,7[illegible] megaloblasts 800, naked nuclei 2,960.
8 P.M., animal found dead. Postmortem examination shows swelling of lymphatic structures and hæmorrhages into them; peritoneal and omental hæmorrhages; small spleen; bone marrow dark red and soft; other organs normal, except for presence of cysticerci pisiformes in peritoneal cavity. In the stained smears of the blood it was noted that before administration of the toxin there was 1% of myelocytes in the circulation, and in the subsequent count all the polymorphonuclear cells showed marked signs of injury in fragmentation of nuclei and karyolysis. One mitotic figure in a megaloblast was encountered.

A similar picture, and evidence in favor of the same view was obtained by giving a second dose of the toxin at the time of the height of the reaction to the first. The protocol is as follows:

Experiment VIII.—Rabbit R. R. M., weight, 1,700 gms.
xi—21—'04. R. b. c. 7,000,000, w. b. c. 10,250.
10.30 A.M., $\frac{1}{40}$ mg. ricin in salt solution injected in ear vein.
xi—22—'04. 10 A.M., r. b. c. 5,584,000, w. b. c. 17,500, normoblasts 1,120, naked nuclei 875 per cu. mm.
11 A.M., $\frac{1}{40}$ mg. ricin injected in ear vein.
8 P.M., w. b. c. 26,700, normoblasts 3,872, megaloblasts 400, naked red-cell nuclei 2,884.
xi—23—'04. 10 A.M., animal found dead. Postmortem examination shows lymphatic structures swollen and hæmorrhagic; many peritoneal hæmorrhages; large soft purplish spleen, measuring 6 x 1 x 0.5 cm.; numerous opaque yellow foci of necrosis in liver; firm dark red bone marrow. Hæmorrhages into thymus and lungs; chocolate colored urine in bladder.

The study of the sections of the marrow from the rabbits dying acutely of ricin intoxication shows an almost uniform picture. There is a depletion of the marrow of many cells and those remaining show marked signs of injury in pyknosis and fragmentation of their nuclei, while there are numerous minute hæmorrhages. The depletion of cells was especially marked in Rabbit R. R. M. (Experiment VIII) which received the two doses of toxin within a short period of time. This condition of the marrow with the appearance of the nucleated red-cell crises in the circulation led the author to the opinion that he was dealing with a blood picture due not only to destruction of red blood cells in the circulation but also to definite toxic injury to the marrow itself, the resulting picture being entirely different from that of a secondary anæmia and resembling more closely that of a primary anæmia. The sharp nucleated red-cell reaction was to be looked upon as a sign of injury to the marrow and not as a sign of regeneration as seems to be currently held. It seemed probable that the ricin destroyed the mature red cells in the marrow and to a certain extent also the normoblasts at the periphery of the red-cell groups and that the marrow responded to the deficiency of red cells with normoblasts and in some cases with megaloblasts, and further that if the marrow was poor in mature red cells and was already supplying a few nucleated red cells to the circulation, the injury due to the ricin would cause a reaction with a larger number of both normoblasts and megaloblasts (Experiments VII and VIII). The naked pyknotic normoblastic nuclei in the circulation are a further evidence of the destructive action of the toxin on the marrow cells.

While this blood picture was entirely different from the clinical picture of secondary anæmias in human beings, it seemed necessary to control the experiments by the production of secondary anæmias in the rabbit, which was most easily to be done by producing hæmorrhage. This was done and although relatively large amounts of blood were taken the response was entirely different in nature from that to the toxin, as is shown by the following experiments.

Experiment IX.—Rabbit A. A., weight 1,850 gms.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| xi—10—'04. | 10 A.M., | r. b. c. | 5,988,000, | w. b. c. | 9,500, | nu. r. b. c. | 0; | bled | 10 | c.c. from marginal ear vein. |
| | 3.30 P.M., | r. b. c. | 5,244,000, | w. b. c. | 11,750, | nu. r. b. c. | 0; | bled | 10 | c.c. |
| xi—11. | 10 A.M. | " | 5,312,000 | " | 7,500 | " " | 15 | " | 10 | " |
| | 12 NOON | " | 4,936,000 | " | 9,250 | " " | 108 | " | 10 | " |
| xi—12. | 10 A.M. | " | 5,312,000 | " | 8,750 | " " | 0 | " | 5 | " |
| xi—13. | 10 A.M. | " | 4,708,000 | " | 12,000 | " " | 0 | " | 10 | " |
| xi—14. | 10 A.M. | " | 3,808,000 | " | 8,000 | " " | 16 | " | 10 | " |
| xi—15. | 10 A.M. | " | 3,608,000 | " | 6,750 | " " | 0 | " | 2 | " |
| xi—16. | 10 A.M. | " | 2,976,000 | " | 13,500 | " " | 0 | " | 15 | " |

| | | | | | | |
|---|---|---|---|---|---|---|
| xi—17. | 12 NOON, r. b. c. | 3,288,000, w. b. c. | 6,500, nu. r. b. c | 13; not bled | | |
| xi—18. | 10 A.M. | " 3,696,000 | " 6,250 | " " 0; bled | 20 | c.c. |
| xi—19. | 10 A.M. | " 2,380,000 | " 9,000 | " " 18 " | 10 | " |
| xi—20. | 10 A.M. | " 2,312,000 | " 9,000 | " " 18 " | 15 | " |
| xi—21. | 10 A.M. | " 2,160,000 | " 5,000 | " " 0 " | 12 | " |
| xi—22. | 12 NOON | " 1,628,000 | " 7,500 | " " 0; not bled | | |
| xi—23. | 10 A.M. | " 2,104,000 | " 8,250 | " " 0; bled | 12 | c.c. |
| xi—24. | 10 A.M. | " 1,960,000 | " 7,000 | " " 14 " | 20 | " |
| xi—25. | 10 A.M. | " 1,916,000 | " 5,750 | " " 0 " | 18 | " |
| xi—26. | 10 A.M. | " 1,576,000 | " 5,000 | " " 0 " | 5 | " |
| xi—27. | 10 A.M. | " 1,912,000 | " 5,000 | " " 0 " | 10 | " |
| xi—28. | 10 A.M. | " 1,486,000 | " 5,500 | " " 11; not bled | | |

Animal killed; weight 1,700 gms. All organs show marked anæmia. Liver is of a light brownish color with the lobules marked off by an opaque yellow periphery; spleen is small, lax, and pale. Bone marrow is light grayish pink in color and quite firm. Other organs appear normal except for anæmia. Tissues hardened in Zenker's fluid.

EXPERIMENT X.—Rabbit A. B., weight 1,800 gms.

xii—1—'04. R. b. c. 7,324,000, w. b. c. 8,000, nu. r. b. c., 0; bled 0.2 c.c.

xii—3. 11 A.M., bled 45 c.c. from marginal ear vein.

| | | | | | |
|---|---|---|---|---|---|
| xii—4. | 10 A.M., r. b. c. | 3,640,000, w. b. c. | 6,000, nu. r. b. c. | 12; bled | 27 c.c. |
| xii—5. | 10 A.M. | " 2,592,000 | " 5,000 | " " 0; not bled | |
| xii—6. | 12 NOON | " 2,540,000 | " 4,750 | " " 18 | |

Experiment was interrupted at this point.

In the marrow of Rabbit A. A. (Experiment IX), both in smear and section, normoblasts were found to be in preponderance, megaloblasts to be quite numerous and to show active signs of proliferation in the numerous mitotic figures present. In the sections the erythrogenetic groups are prominent and large. The experiments seem sufficient to demonstrate that in rabbits as in man a hæmorrhage is responded to by an active orderly proliferation on the part of the marrow, which supplies but a few nucleated red cells to the circulation, by accident as it were, in this respect differing from the response in the case of direct toxic injury. Incidentally it may be noted that in studying the stained blood preparations of Rabbit A. A. (Experiment IX), after the bleeding had been kept up for a week the red cells showed considerable variation in size, were extremely pale, often polychromatophilic, and showed also a marked basophilia.

Following these experiments attempts were made to produce a chronic anæmic condition by the use of ricin, attempts which ere only partially successful owing to the rapidity with which he rabbit establishes a tolerance to the toxin. Anæmias were roduced, however, of sufficient duration to bring about a blood icture almost identical with that seen in the pernicious anæmia f man. Nucleated red cells, chiefly normoblasts, but with n occasional megaloblast, are constantly in the circulation. The red cells vary much in size and within a week after the first injection large non-nucleated red cells, typical macrocytes, form a considerable percentage of the total number. Microcytes also are found and there is a slight tendency to poikilocytosis. This becomes more marked as the anæmia progresses and the cells show polychromatophilia and at times granular basophilia. The picture as far as the leucocytes are concerned differs, however, as there is a constant leucocytosis which is due to an increase of the polymorphonuclear elements, both pseudo-eosinophiles and basophiles. The small mononuclear elements show a relative diminution, but as to their absolute number remain almost constant with an occasional slight rise. The large lymphocyte, however, which is not found in the normal circulation, or if so only very occasionally, becomes a prominent feature of the count, of which it may form as high as 15 or 20 per cent. An occasional myelocyte is found. Three rabbits, R. R. T., R. R. N., and R. R. P., gave almost the same picture and hence it seems necessary to quote but one experiment, that of R. R. T. which was continued the longest. In this animal, with the establishment of tolerance to the ricin and in spite of increasing doses, the red count rose rapidly, passed the normal count, giving a sharp hypererythrocytosis and then returned to the normal, the cells becoming also more uniform in size. The counts are as follows:

EXPERIMENT XI.—Rabbit R. R. T. mottled, weight 1,505 gms.
iv— 6—'05. R. b. c. 6,212,000, w. b. c. 15,500.
2 P.M., intravenous injection of $\frac{1}{60}$ mg. ricin.
iv—10. R. b. c. 5,432,000, w. b. c. 29,000, normoblasts 58.
12 NOON, $\frac{1}{50}$ mg. ricin injected intravenously.
iv—14. R. b. c. 4,788,000, w. b. c. 16,500, normoblasts 33.
11 A.M., $\frac{1}{40}$ mg. ricin injected intravenously.
iv—18. R. b. c. 3,256,000, w. b. c. 44,500, normoblasts 170, megaloblasts 85, naked nuclei 340.

iv—20. R. b. c. 4,296,000, w. b. c. 41,500, normoblasts 1,275, naked nuclei 249.
12 NOON, $\frac{1}{40}$ mg. ricin injected intravenously.
iv—24. R. b. c. 5,044,000, w. b. c. 27,000, no nu. r. b. c.
iv—25. 10 A.M., $\frac{1}{20}$ mg. ricin injected intravenously.
iv—28. R. b. c. 6,820,000, w. b. c. 15,000, no nu. r. b. c.
11 A.M., $\frac{1}{10}$ mg. ricin given intravenously.
v—6. R. b. c. 7,576,000, w. b. c. 9,750, no nu. r. b. c.
11 A.M., $\frac{3}{20}$ mg. ricin injected intravenously.
v—10. R. b. c. 6,340,000, w. b. c. 8,500, no nu. r. b. c.
10 A.M., $\frac{1}{5}$ mg. ricin injected intravenously.
v—15. R. b. c. 6,360,000, w. b. c. 11,500, no nu. r. b. c.
v—26. Animal killed. Peritoneal cavity shows excess of clear fluid. Organs in general normal; spleen small; liver somewhat pale with slightly granular surface; bone marrow of femur dark red and firm.

It does not seem of value to give the differential counts, but a few points are of interest. On iv—10, one myelocyte was found in counting 500 cells, on iv—20, the polychromatophilia and granular basophilia were very marked and the macrocytes became very numerous. Macrocytes were still present in numbers on iv—24 and 28, and many showed paleness. They continued to the end of the experiment in some numbers while in the later smears there were many small poikilocytes found.

As a result of these experiments, the action of ricin on the red cells of the rabbit when injected intravenously may be summarized as one of destruction, affecting not only circulating erythrocytes but also those in the marrow and the erythroblasts in the marrow as shown by the fragmented pyknotic nuclei seen in sections of the marrow and by the naked red-cell nuclei found in the circulation. This destruction results in a diminished red-cell count and in the appearance of large numbers of nucleated red cells, both normoblasts and megaloblasts, in the circulation, and, if the intoxication is continued, in the appearance of pathological forms of erythrocytes in the circulating blood, giving a picture resembling that seen in pernicious anæmia in human beings, and one entirely different from that following severe hæmorrhage.

Following the somewhat disappointing efforts to produce a chronic anæmia in the rabbit with ricin, a series of experiments was made with saponin which is intensely hæmolytic for rabbit blood in the test tube, and to which apparently a tolerance is not so easily established. The toxicity of the specimen of saponin used was from 1.5 to 2 mg. per kilo of body weight for he fatal dose. In the body there was evidence of slight action n the leucocytes, only a few cells with pyknotic nuclei being ound in the circulation. The destructive action on the red ells was shown, as in the ricin poisoning, by reduction in the ed count, by accumulations of pigment in lymphoid organs, and, when injected intravenously, by a prompt appearance of large numbers of nucleated red cells in the circulation, a reaction much more intense than that after ricin poisoning. The nucleated red cells consist of normoblasts, in greatest numbers, megaloblasts and naked pyknotic nuclei. With repeated doses of toxin the blood picture is similar, again, to that following ricin and is strikingly like the blood of a primary anæmia in man. In about a week's time there is a marked variation in size of the red cells, macrocytes being very numerous, there is some poikilocytosis, slight polychromatophilia, and slight granular basophilia, while nucleated red cells are constantly in the circulation. There was in all of the cases counted a marked relative and absolute decrease in the polymorphonuclear elements with a corresponding increase in the mononuclear cells. In this feature saponin differs in its effects from ricin.

The effects of saponin on the blood are well shown by the following experiments.

EXPERIMENT XII.—Rabbit S. R. A., Belgian hare, weight 2,100 gms.

xi—6—'05. R. b. c. 5,120,000, w. b. c. 6,250.

xi—8. 10 A.M., w. b. c. 6,000.
11.30 A.M., intravenous injection of 1 mg. saponin in salt solution.
4 P.M., r. b. c. 4,520,000, w. b. c. 6,750, normoblasts 1,378, megaloblasts 52, naked nuclei 286.
11 P.M., w. b. c. 10,000, normoblasts 6,000, megaloblasts 120, naked nuclei 100.

xi—9. 11 A.M., r. b. c. 5,312,000, w. b. c. 18,000, normoblasts 8,712, megaloblasts 144.

xi—10. 12 NOON, r. b. c. 3,900,000, w. b. c. 10,250, normoblasts 780, megaloblasts 40.
4 P.M., intravenous injection of 1 mg. saponin.
7 P.M., w. b. c. 6,000, normoblasts 936, megaloblasts 24.
12 NOON, w. b. c. 7,000, normoblasts 476, megaloblasts 28.

xi—11. 5 P.M., r. b. c. 3,528,000, w. b. c. 8,375, normoblasts 752, megaloblasts 32.

xi—12. 8 P.M., r. b. c. 2,900,000, w. b. c. 6,000, normoblasts 432, megaloblasts 24.

xi—13. 7 P.M., r. b. c. 2,752,000, w. b. c. 9,250, normoblasts 516.

xi—14. 11 A.M., w. b. c. 7,500, normoblasts 494, megaloblasts 15.

12 NOON, intravenous injection of 1 mg. saponin.

10 P.M., w. b. c. 5,500, normoblasts 847, megaloblasts 33, naked nuclei 66.

xi—15. 10 A.M., r. b. c. 2,724,000, w. b. c. 10,250, normoblasts 2,111, megaloblasts 82, naked nuclei 266.

xi—16. 11 A.M., r. b. c. 2,888,000, w. b. c. 11,000, normoblasts 5,500, megaloblasts 88, naked nuclei 154.

✓ 12 NOON, intravenous injection of 1.5 mg. saponin.

11 P.M., w. b. c. 9,625, normoblasts 4,598, megaloblasts 19, naked nuclei 323.

xi—17. 11 A.M., r. b. c. 2,248,000, w. b. c. 20,000, normoblasts 10,080, megaloblasts 280, naked nuclei 440.

xi—18. 11 A.M., r. b. c. 2,544,000, w. b. c. 10,375, normoblasts 2,520, megaloblasts 60, naked nuclei 80.

✓ 11.30 A.M., intravenous injection of 1.5 mg. saponin.

11.30 P.M., w. b. c. 15,250, normoblasts 1,891, naked nuclei 122.

xi—19. 12 NOON, r. b. c. 3,368,000, w. b. c. 8,375, normoblasts 224, naked nuclei 80.

2 P.M. Animal killed. Organs in general show little change. The spleen is large and dark purplish-red in color; liver and kidneys have a light grayish brown color; lymph glands appear slightly hyperplastic; the bone marrow of the femur is mottled, and firm to the touch, dark red areas of hæmorrhage alternating with firm grayish white areas. There are no other hæmorrhages.

The differential count it does not seem necessary to give in full. It is of interest, however, that on xi—10 at 7 P.M. with a leucocyte count of 6,000, the polymorphonuclear pseudoeosinophiles form but 1.6%, while the small mononuclears are 90.2%. The count at the outset showed 20.2% of polymorphonuclears and 65.8% of lymphocytes. After xi—10 there is a rise in percentage and total number of polymorphonuclears until of the last count on xi—19, they form 55.2% of a count of 8,375, lymphocytes 25.6%.

Rabbit S. R. D. gave almost as sharp a nucleated red-cell crisis as Rabbit S. R. A. Eight hours after an intravenous injection of 3 mg. of saponin, there were in the circulation, 3,102 normoblasts, 85 megaloblasts and 410 naked red-cell nuclei per cu. mm., and 24 hours after, the number had increased to 5,978 normoblasts and 98 megaloblasts per cu. mm. The red count was reduced from 5,248,000 to 3,376,000 in 7 days, when the animal died from an increased dose of toxin. The differential count showed the same relative decrease in the number of polymorphonuclears as was shown in the experiment just quoted, the minimum percentage, however, being 9.4 per cent. a fall from 25.4 per cent.

Rabbit S. R. E. was carried over the longest time and while the blood counts are similar to those above, I quote the experiment as the pathological findings are somewhat remarkable.

Experiment XIII.—Rabbit S. R. E., Belgian hare, weight 2,050.
ii—19—'06. 10 A.M., r. b. c. 5,680,000, w. b. c. 10,250.
11 A.M., intravenous injection of 2 mg. saponin.
ii—23. 10 A.M., r. b. c. 5,040,000, w. b. c. 10,300, nu. r. b. c. 483.
11 A.M., intravenous injection of 3 mg. saponin.
ii—26. 10 A.M., r. b. c. 4,240,000, w. b. c. 8,500, nu. r. b. c. 731.
11 A.M., intravenous injection of 3 mg. saponin.
iii—2. 10 A.M., r. b. c. 4,016,000, w. b. c. 8,375, nu. r. b. c. 1,258.
11 A.M., intravenous injection of 3 mg. saponin.
iii—5. 10 A.M., r. b. c. 3,376,000, w. b. c. 12,250, nu. r. b. c. 1,508.
2 P.M., intravenous injection of 3 mg. saponin.
iii—8. 10 A.M., r. b. c. 3,632,000, w. b. c. 9,000, nu. r. b. c. 738.
11 A.M., intravenous injection of 3 mg. saponin.
iii—12. 10 A.M., r. b. c. 3,876,000, w. b. c. 9,500, nu. r. b. c. 380.
2 P.M., intravenous injection of 4 mg. saponin.
iii—15. 10 A.M., r. b. c. 3,216,000, w. b. c. 14,000, nu. r. b. c. 1,064.
12 NOON, intravenous injection of 4 mg. saponin.
iii—17. W. b. c. 14,000, nu. r. b. c. 2,128.
iii—18. 9 A.M., animal found dead. Organs in general appear normal. The spleen is large and of a dark purplish color; the liver has a granular surface, and on section shows numerous grayish white lines and dots giving a somewhat mottled appearance. The bone marrow is very firm, is largely of a grayish white color mottled with areas of dark red. The mesenteric lymph glands appear small.

In studying the lesions caused by saponin one is struck by the fact that they are confined almost exclusively to the hæmopoietic system, and that the bone marrow is the organ most severely injured. Of the other organs the liver alone shows any marked lesion. The nervous system was not studied. In the bone marrow of a rabbit dying of acute saponin poisoning (Rabbit S. R. F. in which death followed within 48 hours after an intravenous injection of 3 mg. of saponin) the marrow shows partial depletion of mature cells, necrosis to a moderate extent of remaining cells, as shown by pyknotic and fragmented nuclei, and diffuse hæmorrhage. The last is the most striking lesion and was present in all the marrows studied. It seems somewhat remarkable that there should be such extensive hæmorrhage in the marrow when other organs of the body are practically free from it. There are no macroscopical hæmorrhages such as ar the usual lesions in ricin poisoning, nor are microscopical hæmorrhages frequently found. The delicate capillaries of the marrow seem especially susceptible to the poison.

In rabbits which survive repeated doses of saponin for any length of time as Rabbits S. R. A. and S. R. E. (Experiments XII and XIII) there is found a replacement of marrow tissue by a scar tissue composed of fibroblasts, delicate fibrillar connective tissue with a new growth of blood-vessels apparently the result of the organization of the hæmorrhages. In consequence of this the blood-forming cells are limited in distribution and in number. Those present are largely of the mononuclear variety, either in different cells or myelocytes arranged in typical marrow groups. Erythrogenetic groups are well marked.

The sclerosis and replacement of the marrow were most marked in Rabbit S. R. E. which had survived the initial dose of saponin one month, and the lesion was so extreme that at first it seemed difficult to account for the maintenance of the red count of the blood above 3,000,000 cells per cu. mm. It was only explained after examination of the spleen in this rabbit and in others of the series. In this case the peripheral venous sinuses of the spleen were much dilated and crowded with cells of the marrow type chiefly of the erythrogenetic series, but including many megalokaryocytes and leucocytes. The nucleated red cells were grouped much as in the marrow and showed numerous mitotic figures. A similar but less extensive picture was present in Rabbit S. R. A. and in fact in the whole series; marrow cells and in particular in several cases the megalokaryocytes were very numerous in the splenic sinuses. The lesion is so definite and striking that there is no question but that one is dealing here with vicarious formation of blood cells in the splenic sinuses, a picture similar to that recently described by Meyer and Heineke[2] as occurring in severe anæmias which the author, also, has observed in the spleen of a case of pernicious anæmia. The veins of other organs are practically free from nucleated blood cells, except for an occasional small group in the liver and the constant p of megalokaryocytic nuclei in the capillaries of the lung.

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[2] *Verhandlungen der Deutsch. Path. Gesellschaft,* 1906, ix, 224.

The liver in the acute cases, with the employment o doses of toxin, shows a central necrosis of the lobules marked turgescence of the veins and capillaries. In S. R. E. were found the results of this necrosis in nu recent scars showing fibroblasts and delicate connective on the border of which were numerous groups of sma cells with basophilic protoplasm, apparently recently f and in fact giving direct evidence of the regenerative pro numerous mitotic figures. The scars lie chiefly within the and are independent of the portal spaces.

To summarize the effects of saponin intravenously istered, they are a destruction of red blood cells in the circ and to a less extent of the leucocytes, a destruction of the marrow and in particular an injury to the capillar resulting in extensive hæmorrhages. The circulating blood a reduction in the red-cell count, and sharp nucleated crises, and later, pathological changes in the erythrocytes. result of the marrow hæmorrhages there is a sclerosis marrow which may almost completely replace the hæmo cells. The production of blood elements may then be on in the sinuses of the spleen.

It will be noticed that in the experiments thus far repo this paper, the toxins have uniformly been injected intrave and that there has been a uniformity in the results wit ricin and saponin in the injury of the marrow, and the re blood picture seems from other experiments to be entir pendent upon the presence in the circulating blood of su toxin to reach the marrow and produce injury and necrosi cells. It is not given when toxins are injected subcuta or intraperitoneally unless in large doses. In the whol of rabbits treated with hæmolytic, myelotoxic, and lymp sera intraperitoneally, the results of which were reported previous paper,[3] only exceptionally was a single norr encountered in making a differential count of 500 leucocyt the marrow picture as far as the erythrogen concerned was the hyperplastic picture seen i anæmia resulting from hæmorrhage, and at the accumulations of pigment in the spleen pulp evidence of extensive blood destruction.

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[3] *Op. Cit.*

With the intraperitoneal injection of $\frac{1}{10}$ milligra of ricin of which $\frac{1}{30}$ milligram was a fatal dose w the blood picture was quite different from *that* intravenous injection. There was a more *prom* reaction, and fewer of the leucocytes showed *sig* the nucleated red cells at the end of 24 hours were cubic millimeter, a much smaller number than *with* injection of smaller amounts, as shown by *experiment* quoted. On examination of the marrow postmorten found very slight injury — a very few *fragmented* practically no hæmorrhages. The counts are as *follc*

Experiment XIV.—Rabbit R. R. K., weight 1,650.
xi—10—'04. R. b. c. 8,252,000, w. b. c. 11,250.
10 A.M., $\frac{1}{10}$ mg. ricin in 0.83% salt solution *injected intrape*
2 P.M., w. b. c. 37,000, no nucleated red cells.
8 P.M., w. b. c. 23,000, no nucleated red cells.
xi—11. 10 A.M., r. b. c. 8,468,000, w. b. c. 19,500, normoblasts 35
nuclei 351.

Rabbit died at 2 P.M. Peritoneal cavity contains excess of *clea* fluid. There are a few minute hæmorrhages in the omentum. *The* enlarged and of a dark purplish color. The mesenteric lymph glands a swollen, and hæmorrhagic with peripheral light opaque areas, *evidentl* of necrosis. Bone marrow firm grayish red.

Here there was apparently a dose of sufficient *intensit* affect the marrow somewhat, though with nothing *of the seve* of the intravenous injection of a dose of the same *strength,* the nucleated red-cell reaction is consequently *much less, w* the leucocytic reaction is more marked.

With subcutaneous injections of doses of saponin *which intr* venously call out several thousand erythroblasts one *finds n* nucleated red cells in the circulation 24 hours later, and the sam result follows repeated injections. The bone marrow postmortem shows also no hæmorrhages, and has a hyperplastic appearance.

Similar blood pictures were given by Rabbits S. R. G. and S. R. H. The counts in the latter are as follows:

Experiment XV.—Rabbit S. R. H., Belgian hare, weight 1,900 gms.
v— 4—'06. R. b. c. 5,432,000, w. b. c. 6,500, no nu. r. b. c.
12 noon, 3 mg. saponin injected subcutaneously.
v— 5. 11 A.M., w. b. c. 13,500, no nu. r. b. c.
v— 7. 10 A.M., 3 mg. saponin injected subcutaneously.
v— 8. 10 A.M., w. b. c. 18,000, no nu. r. b. c.
v—10. 10 A.M., r. b. c. 5,552,000, w. b. c. 17,500, no nu. r. b. c.

Animal killed. Postmortem an inflammatory reaction is found at point of one injection; the spleen is large dark purplish in color; lymph glands are hyperplastic; bone marrow is fairly soft, of a grayish red color, quite opaque, and shows no hæmorrhages. Kidneys show numerous white streaks and dots in the cortex but no definite abscess formation.

In this rabbit although there was no reduction in the blood count, there was found definite microscopical evidence of blood destruction in the accumulations of light yellow blood pigment in the spleen, and the marrow, which was apparently entirely uninjured by the toxin, showed marked hyperplasia, the erythrogenetic centres being well marked and showing many peripheral normoblasts. Undoubtedly the marrow had thus far been able to compensate for the destruction of circulating cells. Rabbit S. R. G. after two similar subcutaneous injections of 4 mg. of saponin gave a fall of 1,000,000 red cells in the count in a week without the appearance of nucleated red cells in the circulation. Subcutaneous injection of the exquisitely hæmolytic toxin isolated by Dr. Ford from Amanita phalloides gave a similar negative blood picture as far as the nucleated red cells were concerned. From quite an extended series of animals, it may be said, that unless the toxin is given subcutaneously in doses so large that after its entrance into the circulation it is not neutralized entirely by the circulating blood cells, one will not get injury of the marrow or nucleated red-cell crises, but rather a compensatory hyperplasia of the marrow, similar to that following hæmorrhage, in which orderly erythrogenetic groups with many normoblasts are the rule, the marrow in other words of a secondary anæmia.

It seems justifiable to conclude from these experiments in addition to the anatomical features described at the outset that:

(1) Nucleated red-cell crises in the circulating blood are the

expression of injury to the bone marrow
reacts with nucleated cells only when the
at the periphery of the erythrogenetic centr
by the action of a circulating toxin, or are d
hæmorrhage. In the first case the reaction is
than in the second.

(2) Following toxic injury to the marrow
the peripheral cells of the erythrogenetic gro
lished an atypical formation of erythrocytes
logical forms, the most characteristic of which a
These appear to be directly derived from the me
the orderly transition through the intermediate
stages. This condition may represent an attem
tory repair.

(3) Following extensive injury to the marro
the groups of blood-forming cells almost complet
scar tissue. The spleen may then take on th
function, the new cells being formed in the sinuses

(4) With subcutaneous injections of hæmolyt
absorption is so slow that the toxin is apparent
by cells in the circulation, and does not reach
in sufficient quantity to injure it. Under thos
there is blood destruction, but no nucleated red
in the circulation, and the marrow picture is one of
such as is seen after hæmorrhage, *i.e.*, the marrow of a
anæmia.

# AN IMPROVED SYRINGE FOR INJECTING PRECISE AMOUNTS.*

By ARTHUR PARKER HITCHENS, M.D., Glenolden, Pa.

Plate XLI.

The method described by Rosenau[1] for injecting precise amounts of fluids into animals is excellent. In the practical application of this method certain difficulties have however been encountered, which, it is believed, can be overcome by improvements in the syringe herein described. The time required for removing the bulb of and measuring the salt solution into the syringe to wash it out, after the inoculating fluid has been injected, is considerable and requires the aid of an assistant. Moreover, the cost of the syringe makes it a rather expensive device when a number of these are required, such as are used for determining the strength of toxins and antitoxins.

The improvements in the syringe which I have devised consist of a side arm on the barrel of the syringe and a flexible rubber joint between the barrel and the needle. The value of this side arm is stated below. The flexible rubber joint obviates the necessity of having threads moulded on the lower end of the barrel and allows the tilting of the syringe when in use.

*The Syringe.*—In the text figure, the various parts of the syringe are clearly depicted. The glass portion is made of tubing, the inside diameter of which is from 10 to 12 millimeters. The total length of the barrel is 100 millimeters. The upper end is flared, to admit the stopper of the rubber bulb; and lipped. At the other end, a small neck (about 3 mm. in diameter), 15 millimeters long, is made to receive the rubber tubing which holds the needle.

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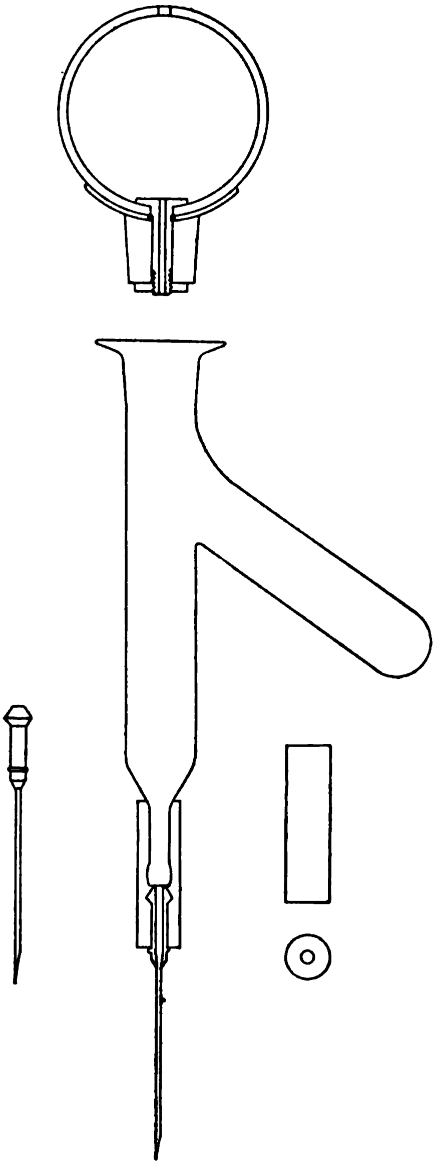
*Read at a meeting of the Society of American Bacteriologists, Ann Arbor, Dec. 28–29, 1905.

[1] Bull. No. 19, 1904, Hyg. Lab. U. S. Pub. Health & Marine Hosp. Service, Washington, pp. 1–7.



A bulging near the extremity of the neck prevents any tendency on the part of the tubing to slip off.

About the point where the neck joins the shoulder the calibre should be rather small. Thus when different fluids are measured into the syringe, the first does not trickle down into the needle, preventing its mixture with the other fluids, but is held up by capillary attraction.

The rubber connection is made of heavy walled, pure gum tubing; it is 32 millimeters long by 8 millimeters outside diameter, and the caliber is about 1.5 millimeters. The rubber bulb is essentially the same as that of the Koch syringe. It should be fairly heavy and of rather inelastic rubber. The needle is made of "19 gauge" tubing and exclusive of the hub is 40 millimeters long.[2]

Any glass blower who is able to make a "Y" tube should have no trouble in making the glass portion of this syringe.

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[2] For making subcutaneous injections into guinea pigs, the writer prefers the needle dulled or with a chisel point. When the abdomen of the animal is not shaved and the injection is made transversely, it is not easy to keep a very sharp pointed needle in the subcutaneous tissues.

*Method of Use.*—The upper end of the glass portion is plugged, and the lower end wrapped with cotton. It is then sterilized by dry heat. The needle and rubber connection always remain together and just before using are boiled in 1 per cent. borax solution.

When the syringe is to be put together, the cotton is removed from the lower end of the glass barrel and the rubber, still hot and moist (from which the solution has just been drained), is slipped on, up to the shoulder, thus bringing the needle and syringe into apposition.

The rubber when cool is quite dry and clings firmly to the glass. The wire being removed, the needle is then dipped into white petrolatum to seal it.

The requisite number of syringes, one for each animal, is thus prepared and placed in racks. The fluid to be injected is measured into the barrel of the syringe and a definite quantity of saline solution is put into the side arm.

When an injection is to be made, the cotton stopper is first taken out and the bulb fitted into its place. The syringe is held as shown in Plate XLI, Fig. 1. The little finger supports it in the angle at the junction of the arm and the barrel, and the rubber connection is grasped firmly just over the hub of the needle between the thumb and first and second fingers.

After the fluid has been injected in the ordinary manner, the syringe is either rotated on its long axis or tilted, the salt solution runs into the barrel from the arm and is in turn injected into the animal. This obviates the necessity of the removal of the bulb and the addition of the saline solution by another person—procedures which more than double the time necessary for a simple injection, and require the services of an extra assistant.

Rarely a needle may become occluded so that the rubber bulb is not strong enough to force it open. To overcome this difficulty, wire of platinum with 10 per cent. iridium, to make it less liable to bend, has been fitted into the end of an aluminum rod after the fashion of the Ravenel style of inoculating needle. This is sterilized and run down through the syringe and needle without removing the latter from its position in the tissues of the animal.

After having been used, the syringe is taken apart and the wires are inserted into the needles, which are boiled in borax solution. The borax water is drained off while it is still hot and the needles put away practically dry. The durability of the rubber tubing when so treated is surprisingly long. A number of pieces have been in almost constant use during the last five months and their elasticity seems in no way impaired. The glass portions are soaked first in sulphuric acid and potassium bichromate, and then in soda solution. After having been rinsed thoroughly, they are allowed to drain before sterilization.

The racks hold twelve syringes.[3] The holes in the cross piece at the top are so slanted that when the arm is put through, the barrel of the syringe is held perpendicular and swings free.

The ground-glass shelf, suggested by Rosenau, has been used for the reasons which he notes. Plate XLI, Fig. 2 gives a rear view of the rack with its complement of syringes. The syringe resting on the shelf is intended to show the manner in which the glass portion is prepared for sterilization.

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[3] The racks may of course be made to hold any number of syringes. One holding a dozen seems the most convenient size.

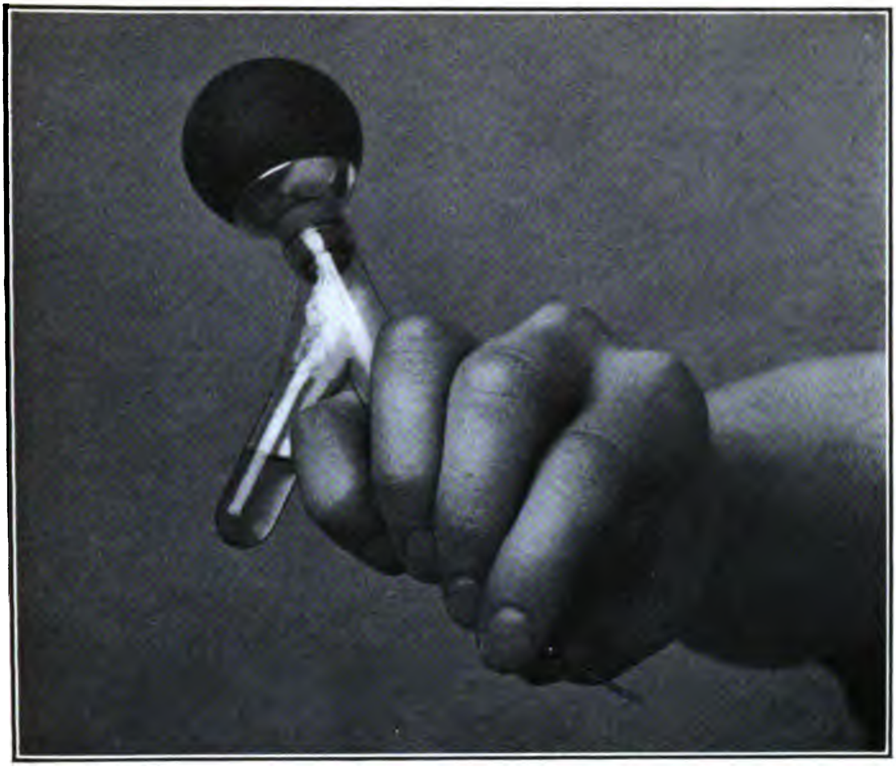
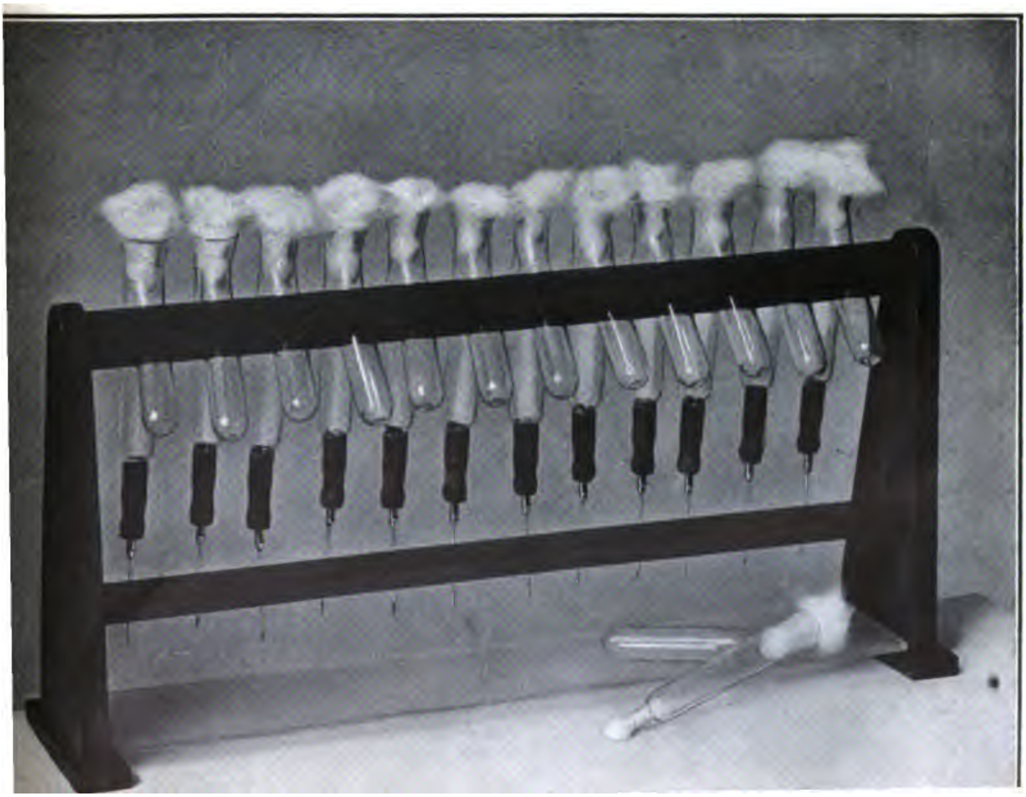


Fig. 1.



# A CONTRIBUTION TO THE STUDY OF THE OPSONINS.*

By CHARLES E. SIMON, M.D., in Association with R. V. LAMAR, M.D.,
AND
W. N. BISPHAM, M.D.

(*From the Laboratory of Dr. Charles E. Simon.*)

Received for publication April 2, 1906.

Through the researches of Wright and Douglas[1] it has been established that the phagocytic effect of human leucocytes upon certain bacteria is essentially dependent upon the presence in the blood plasma and serum of substances which they have termed opsonins. They could demonstrate that these substances *per se* are to a certain extent at least thermolabile, being partly destroyed by heating the blood serum to a temperature of 60-65° C. for ten minutes or more. The phagocytic effect of such serum is accordingly diminished and may indeed be suspended. If, however, bacteria are first digested with normal serum and subsequently exposed to a temperature of 60–65° C. phagocytosis proceeds in a normal manner. The organisms in question are the staphylococci, Bacillus pestis, Micrococcus melitensis, Diplococcus pneumoniæ (Fränkel), Bacillus coli, Bacillus dysenteriæ, Bacillus anthracis, Bacillus typhosus, Bacillus tuberculosis and the vibrio of Asiatic cholera, while the diphtheria bacillus and Bacillus xerosis were found to be insensible to opsonic action. With the latter phagocytosis proceeds as promptly with heated as with unheated serum.

In a series of further studies Wright and his collaborators then illustrate the variations in the opsonic content of the blood in certain infections and discuss the significance of the data obtained from the standpoint of immunity. The therapeutic inferences to which they are led and the practical results which

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* Conducted under a grant from the Rockefeller Institute.

[1] Wright and Douglas, *Proc. Royal Soc.*, 1903, lxxii, 357; *ibid.* 1904, lxxiii, 128; *ibid.*, 135, and *ibid.*, 147.

they claim to have obtained are highly suggestive and have attracted wide-spread attention.

The studies described in the present paper were undertaken with the idea of gaining a further insight into the nature of the opsonins and their significance from the standpoint of immunity.

## TECHNIQUE.

For the purpose of estimating the phagocytic power of the leucocytes Wright makes use of a modification of the method originally devised by Leishman. To this end a sample of blood is drawn off and mixed with one tenth of its volume of a ten per cent. solution of sodium citrate. A second sample of blood is allowed to clot in the usual way. In the case of the first sample the corpuscles are isolated from the plasma by repeated washing with physiological salt solution and centrifugation; washed corpuscles are thus obtained. In the case of the second sample the serum is simply separated from the corpuscles in the ordinary way by centrifugalization. Equal parts of washed corpuscles (two or three) and serum are then taken and further mixed with one volume of a suspension of the organism under consideration, obtained by rubbing up in physiological salt solution a portion of a twenty-four hours growth on agar. The resultant mixture is incubated at blood heat for fifteen minutes, when smears are made and stained with Leishman's stain. Finally the number of ingested bacteria is counted in a series of polynuclear leucocytes, taken in the order in which they are observed, and the average for one leucocyte calculated; the resultant figure denotes the phagocytic power. This is further compared with that of a normal control person, which latter serves as unit. The ratio between the two values is termed the opsonic index of the blood.

This method has thus far been employed almost exclusively in the study of the opsonins, and upon it the results obtained by Wright and his co-workers are based.

In some of the earlier work which was carried on by one of us (Simon) before the present studies were undertaken, the original method of Leishman was used. To this end equal parts of blood and bacterial suspension are mixed and a drop of the mixture placed on a slide, covered with a coverglass and incubated at 37° C.; slide and cover are drawn apart and the resultant smears stained as above. The average number of organisms pro leucocyte is then determined as with Wright's method. For purposes of class demonstration of phagocytosis this method was found quite satisfactory, but it does not appear to us that either in the original form or in that now employed by Wright the mere determination of the average number of organisms pro leucocyte gives an adequate idea of the quantity of opsonins present. It is thus quite conceivable that in a given case the use of concentrated serum may show what would appear to be a perfectly normal and sufficient quantity of opsonins for a given number of organisms, which amount, however, might be altogether inadequate for a larger number. This inadequacy would become manifest at once upon diluting the serum, when a rapid exhaustion of the phagocytic power would occur. Our own investigations have shown conclusively that such an event is by no means uncommon. Serum of the pig in concentrated condition thus manifests a most intense opsonic effect for staphylococci, which diminishes with marked rapidity, however, on dilution, while with human serum the dilution can in most cases be carried much further, even though the initial phagocytic power is less. Similarly we find that with certain organisms, such as the anthrax bacillus, the colon bacillus, Fränkel's pneumococcus etc. the number of organisms ingested by a leucocyte is relatively small, but that the same serum can be quite freely diluted before phagocytosis ceases. We have found in human beings who were supposedly in good health that a good phagocytic power in concentrated serum may diminish on the one hand in proportion to the degree of dilution, while on the other rapid exhaustion may take place. Under such circumstances we can hardly find it warrantable to conclude that the opsonic content in the two mixtures is identical.

But apart from these considerations there are other reasons which render the determination of the opsonic content according to Wright's method inadvisable for general work. With the use of concentrated serum it may thus be absolutely impossible to count the number of staphylococci in a given cell. With normal blood or in cases where the phagocytic effect is diminished this difficulty is not so great, but in infections in which the opsonic power is increased enumeration is out of the question. The same holds good when working with the blood serum of certain animals, such as the pig. The number of organisms which are here taken up in concentrated serum is most remarkable; the cells are filled to bursting and accurate counting is impossible. With other organisms there are other difficulties. In the case of the typhoid bacillus, the colon bacillus, the bacillus of Asiatic cholera, and Metchnikoff's bacillus spherulation of the organisms and loss of staining power commonly occur and frequently preclude all possibility of obtaining a satisfactory result by counting. The agglutinating effect of certain sera upon the corresponding organisms is a further drawback to the method, which presupposes that the emulsion of organisms to be added to the blood serum should be uniform and that no agglutinative effect should precede phagocytosis. This difficulty is of special importance when dealing with the tubercle bacillus, and has not yet been satisfactorily overcome.[2] In the case of the anthrax bacillus—and the same applies to Bacillus subtilis—enumeration is for obvious reasons inapplicable and may indeed be impossible.

Led by these considerations, among others, we abandoned the idea of determining the opsonic content of the blood according to the Leishman-Wright method, and have devised the following method, which we believe is more satisfactory and free from the objections which attach to the older procedure.

*Method.*—By means of a suitably bent and drawn-out glass tube about one half a cubic centimeter of blood, which is readily obtained by the usual puncture of the finger or preferably the ear, is transferred, in portions, to a small tube and mixed with a large excess of a 1.2 per cent. solution of sodium chloride and 0.1

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[2] With the method advocated by Wright we could not obtain a satisfactory emulsion of tubercle bacilli. Through the kindness of Dr. E. R. Baldwin of the Saranac Research Laboratory for the study of tuberculosis we were furnished with a supply of bacilli which had been extracted with chloroform. With these much better results were obtained, the bacilli being rubbed up in an agate mortar with 1:1000 salt solution and then centrifugalized. We take pleasure in acknowledging the courtesy of both Dr. Trudeau and Dr. Baldwin.

per cent. of ammonium oxalate. The tube is filled with the same solution and blood and diluent are well mixed and centrifugalized. When the corpuscles are well packed down the supernatant fluid is drawn off with a small pipette and replaced with 1.2 per cent. salt solution, containing no oxalate. The tube is repeatedly inverted and the corpuscles again thrown down by centrifugalization. The washing is repeated once more and the fluid withdrawn down to the corpuscles. The leucocytes which have been thus washed are entirely inactive. In our experiments they were always obtained from supposedly normal individuals and not kept longer than two or three hours, though they may be serviceable for a longer time. Wright and Douglas state that in their experience there was no indication of a variation of the phagocytic power within the space of a few hours, while after the lapse of three days it had declined to less than one half or one third of that of the freshly drawn blood.

The specimen of blood in which the opsonic content is to be determined (about one half cubic centimeter) is collected in a similar manner, but without the use of a diluent. It is placed in a small *dry* tube and allowed to clot, after which the clot is carefully separated from the walls and packed down by centrifugalization. A large drop of the serum which separates out is placed upon a slide and from this various dilutions are made with 1.2 per cent. salt solution. With human blood we usually prepare a 1:20, a 1:30 and a 1:40 dilution, while with the blood of animals a 1:10 and a 1:20 specimen is made. The dilutions are conveniently obtained by the aid of the Thoma-Zeiss pipette used in counting white blood corpuscles. For routine work we use a volume of diluted serum corresponding to eighteen divisions of the pipette. In the case of the 1:30 dilution we thus take twelve divisions of the 1:20 specimen and six of salt solution and for the 1:40 nine of the 1:20 and nine of the salt solution. The resultant preparations are then inoculated with a small amount of the organism under consideration; this is taken from an agar culture and gently rubbed against the tube so that a slight milky turbidity results. If an organism is used which does not readily yield an emulsion in this way, an emulsion must be separately prepared in normal salt solution, or as in the case of the tubercle bacillus in a 1:1000 solution of salt and a small volume of this emulsion is added, due allowance being made for the degree of dilution.[3] Each tube finally receives a constant volume of corpuscles which equals one half that of the diluted serum. The specimens are gently agitated and placed in the incubator at 37°—40° C. for thirty minutes, when large drops of the fluid are placed on slides, spread in the usual manner, (rather thickly) and allowed to dry in the air. It is advisable that in spreading the second slide be merely kept in contact with the blood, without really touching the lower slide; otherwise it may happen that most of the leucocytes, containing organisms, are carried to one end, while only the empty cells are found in the intervening space. When dry the smears are fixed by gently warming them over a flame, when they are stained with an aqueous solution of methylene blue. This has the advantage that in the relatively thick specimens the red cells are not colored and the leucocytes accordingly are more readily found. The use of polychrome dyes is in our experience not at all advantageous.[4] The preparations are finally allowed to dry, when the percentage number of phagocyting cells is determined by counting fifty cells which are taken in sequence. When phagocytosis is slight and doubt arises whether in a given cell an organism lies in or upon a cell it is advisable to go over a larger number of cells. With the higher dilution and slight phagocytosis a certain amount of practice is necessary before constant results can be obtained, and even then the personal factor will enter into consideration; this, however, is less disturbing than with the Leishman-Wright method. The results are expressed as in the following example:

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[3] For routine work we used equal parts of a 1:10 dilution of serum and of the tubercle emulsion, thus obtaining a 1:20 dilution.

[4] With the tubercle bacillus Gabbett's method was used.

| | |
|---|---|
| 1:20 | 100% + |
| 1:30 | 92% + |
| 1:40 | 64% + |

Incidentally note is taken in a general way whether many organisms or only a small number are present in the cells.

A problem that gave us much consideration was the selection of the organism for inoculation. As this involves the question of the specificity of the opsonins we shall deal with it under a separate heading. At this place it will suffice to state that for routine work Staphylococcus citreus was employed.

One point upon which we wish to dwell in this connection and which does not seem to have been noticed by previous observers is the necessity of using a time limit during which the tubes are kept in the incubator. In our experiments this was uniformly thirty minutes. If a longer time is allowed the results may be totally different. Generally speaking the number of phagocyting leucocytes increases with prolonged exposure. In one instance the values with the 1:20 dilution were thus 50 after thirty minutes and 80 after two hours.

## OCCURRENCE OF OPSONINS IN VERTEBRATES.

Hektoen and Rüdiger[5] have shown that among mammals the opsonins of one species will sensitize bacteria for phagocytosis by the leucocytes of a different species. The animals examined were the guinea pig, rabbit, dog, goat, white rat, and horse. To this list we can add the cat, the sheep, the calf and pig. The serum of all these animals was found to sensitize Staphylococcus citreus for phagocytosis by human leucocytes. We have also found that the same holds good for the serum of the chicken, the terrapin, the frog, and the fish—in other words for all the large classes of vertebrates. The amount of opsonin present in the serum of the various animals is variable, however, as determined by the degree of dilution, and seems to be lower as we descend in the scale of animal life (Table I). The degree of phagocytosis, viz. the number of organisms pro leucocyte, is variable, moreover, not only among the representatives of different species, but also

[5] Hektoen and Rüdiger, *Journ. of Infect. Dis.*, 1905, ii, 128.

among individuals (Table II). To a certain extent these differences may depend upon conditions of nutrition and the process of digestion (*vide infra*), but we feel certain that there are still other factors which are of moment in this connection.

TABLE I.

SHOWING THE OPSONIC VALUES OF ANIMALS OF THE DIFFERENT CLASSES OF VERTEBRATES.

| | 1:1 | 1:10 | 1:20 | 1:30 |
|---|---|---|---|---|
| Fish | active | — | — | — |
| Frog | active | — | — | — |
| Turtle[6] | 1:5 = 52 | 40 | — | — |
| Chicken | 56 | 0 | 0 | 0 |
| Guinea pig | | 20 | 12 | 0 |
| Rabbit | | 22 | 10 | 0 |
| Calf | | 36 | 12 | 0 |
| Sheep | | 16 | 0 | 0 |
| Pig | | 96 | 14 | 0 |
| Dog | | 32 | 20 | 0 |

TABLE II.

SHOWING THE VARIABILITY OF THE OPSONIC VALUE IN DIFFERENT ANIMALS OF THE SAME SPECIES (ON FIRST EXAMINATION).

| | | 1:1 | 1:10 | 1:20 | 1:30 |
|---|---|---|---|---|---|
| Rabbits | (122) | | — | 26 | 20 |
| | ( 91) | | — | 2 | 0 |
| | (123) | | 16 | 0 | 0 |
| | (131) | | 2 | 0 | 0 |
| | (132) | | 40 | 40 | 28 |
| | (145) | | 12 | 0 | 0 |
| | (162) | | 72 | 48 | — |
| | (171) | | 4 | 0 | 0 |
| | (188) | | 8 | 0 | 0 |
| Dogs | (182) | | 40 | 28 | — |
| | (214) | | 24 | 2 | 0 |
| | (228) | | 4 | 0 | 0 |

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[6] With the undiluted serum of the turtle there occurs a most extensive agglutination of cocci, with hæmolysis of the red cells and agglutination of the stromata. Under the circumstances it was impossible to obtain any adequate idea of the extent of phagocytosis. On diluting the serum there was still marked agglutination of the cocci, but there was no evidence of hæmolysis.

| | | 1:1 | 1:10 | 1:20 | 1:30 |
|---|---|---|---|---|---|
| Pigs | (89) | 96 | 14 | 0 | 0 |
| | (108) | | 48 | 16 | 4 |
| | (149) | 100 | 8 | 0 | 0 |
| | (202) | 96 | — | — | — |
| | (240) | 96 | — | 10 | — |

Rabbit 91 for example is a large buck in excellent condition, with an initial phagocytic value of 2 with a 1:20 dilution, while Rabbit 132 is a much smaller animal and exhibits a phagocytic value of 40 with the same dilution. Dog 228 on the other hand was received in very poor condition and had a phagocytic value of 4 as compared with two other dogs who showed 40 and 50 respectively with a dilution of 1:10, and were much better nourished.

## THE OPSONIC CONTENT IN HEALTHY HUMAN ADULTS.

As in the case of individual animals of a given species, so also does the opsonic content of the blood vary in man. But human blood generally speaking contains rather more opsonins than the blood of the lower animals which we have examined.

### TABLE III.

TABLE SHOWING NORMAL VARIATIONS OF THE OPSONINS AS ILLUSTRATED BY THE FINDINGS IN TWENTY NORMAL INDIVIDUALS.

| | 1:20 | 1:30 | 1:40 |
|---|---|---|---|
| Dr. B. | 16 | 2 | 0 |
| Dr. D. | 44 | 28 | 12 |
| Dr. K. | 44 | 24 | 12 |
| Dr. S. | 20 | 16 | 4 |
| Mrs. S. | 12 | 8 | 0 |
| Dr. B. | 44 | 24 | 18 |
| Dr. L. | 56 | 32 | 20 |
| M. M. | 72 | 42 | 12 |
| Dr. E. | 44 | 20 | 8 |
| Dr. C. | 48 | 28 | 12 |
| Dr. J. | 56 | 20 | 16 |
| Dr. F. | 18 | 10 | 6 |
| Dr. M. | 42 | 28 | 10 |
| C. | 12 | 6 | 3 |
| Dr. B. | 54 | 24 | 8 |
| H. B. | 12 | 4 | 0 |
| Dr. R. | 68 | 36 | 24 |
| Dr. T. | 24 | 8 | 4 |
| Dr. S. | 20 | 16 | 12 |
| Dr. M. | 38 | 12 | 2 |

The values obtained in a series of twenty normal individuals are given in Table III. From this table it will be seen that with a dilution of 1:20 there is phagocytosis in all, the average being 37.2, with 72 as maximal and 12 as minimal values. With a dilution of 1:30 there is also phagocytosis in all, but already diminished in extent, the average being 19.4. With a dilution of 1:40 (average, 9.2) there is a certain percentage of negative results, which increases with the higher dilution of 1:50. Beyond this it is exceptional to find phagocyting leucocytes.

The figures in the table show that the opsonic content of normal blood fluctuates much more than the determination of the opsonic index according to Wright's method would lead one to believe.

In the following apparently normal individuals high values were found.

| | 1:20 | 1:30 | 1:40 | 1:50 |
|---|---|---|---|---|
| Dr. K. | 100 | 100 | 100 | 63 |
| Dr. J. | 98 | 92 | 48 | 36 |
| Dr. E. | 88 | 40 | 40 | — |
| Dr. A. | 84 | 52 | 20 | — |
| Dr. L. | — | 82 | 18 | 4 |

Dr. K. was an individual who was rather a large eater; hardy and vigorous, with no sign of any infection.

Dr. J. was a person of fairly large build, and well nourished.

Dr. E. was a female physician of light build; a very active person.

Dr. A. is of small stature and does not make the impression of unusual vigor. This value was obtained an hour after breakfast.

Dr. L. is a stout man, who is exceedingly prone to pyogenic infections. At the time of examination he was healthy and well. The finding in his case is certainly opposed to Wright's idea that infection takes place because of a low opsonic content.

Our "normal" series includes a few individuals with very low values, which were not included in the table above, as the persons did not make the impression of being in normal health. Dr. N. thus gave 4 for 1:20 and 0 for 1:30 and 1:40 and Dr. B. 6 and 0 respectively. There was no evidence of any actual illness in either, but both looked pale and were rather thin.

All the individuals named in the table were in good health at the time of examination and would hence according to Wright and Bulloch[7] have shown practically the same index, viz. one varying between 0.8 and 1.2. Supposing this to have been the case it is difficult to understand why in one individual the serum could be diluted fifty times and still retain a certain degree of activity, while in another individual the limit of opsonic power was practically at 1:30, unless we assume, as we do, that the amount of opsonins in the first case was materially greater than in the second.

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[7] Bulloch, W., *Lancet*, 1905, ii, 1605.

In a general way the phagocytic power diminishes in proportion to the degree of dilution, but it is noteworthy that in some individuals it is more rapidly exhausted than in others. Under pathological conditions we meet with the same phenomenon, and not infrequently to an accentuated degree.

While the normal range of phagocytic power with a dilution of 1:20 lies between 12 and 72, remarkable exceptions are at times encountered, as in the case of Drs. K., A., and J. and Miss E. It will be observed that in Dr. K. phagocytosis was still active with a dilution of 1:100, being well maintained from 1:20 down. This same good maintenance is seen in Dr. J. and Miss E. We are not prepared to explain these remarkable exceptions. Such findings in our experience are obtained essentially under pathological conditions, but in the instances mentioned there was no indication of the existence of an infection.[8]

The individual variations may in part be due to differences in the state of nutrition, but are not necessarily proportionate to the body weight (Table III). As will be shown in the following section the opsonic content is to a certain extent dependent upon the process of digestion, but in the above series the individual differences cannot be accounted for upon this basis alone, as the examinations in most cases were made in the afternoon about one or two hours after the midday meal.

The values fluctuate to a certain extent from day to day, but remain within the limits indicated.

We repeat at this place that our results have reference to a time limit of thirty minutes in the incubator and that higher values are obtained if this is materially exceeded.

## INFLUENCE OF DIGESTION UPON THE OPSONIC CONTENT.

In some individuals a marked influence of the process of digestion upon the opsonic content of the blood can be readily established.

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[8] These cases have been separately placed in the above table.

The initial opsonic value of K. at 3 P.M. with a dilution of 1:40 was 100. The same individual after fasting through the night, at 7.30 A.M., showed only 8 per cent. of phagocytosis with a dilution of 1:30. The results at 2 P.M., one hour after a hearty dinner, were the following:

1:30 = 98 1:40 = 96 1:50 = 96

Two hours later the corresponding figures were:

1:30 = 86 1:40 = 74 1:50 = 26

The rapid exhaustion as compared with the results obtained one hour after dinner, is here strikingly shown. The same is seen early in the morning. In the given experiment a 1:20 determination was not made at that hour, but on another occasion the value was 82, and fell to 2 with a dilution of 1:40.

At 6.50 P.M. supper was had and one hour later higher values are again found, which are well maintained with the higher dilutions, viz.:

1:30 = 98 1:40 = 98 1:50 = 98

The figures given in conection with this experiment should not be regarded as averages, however, as they were obtained in the case of Dr. K., who showed an exceptionally high phagocytic value.

The same general result was obtained in the case of a rabbit.

The initial phagocytic value was 4 per cent. for 1:30, and negative for 1:40. After fasting absolutely for 43 hours there was no phagocytosis whatever with 1:30; with 1:20 only 2 per cent. of the cells were positive, and with 1:10 only 20 per cent. The animal was then well fed and about one hour later gave the following values:

1:30 = 28 1:40 = 22 1:50 = 6

Dr. Amberg has made a number of opsonic determinations according to our method in breast-fed babies in the obstetrical department of the Johns Hopkins Hospital. He will report the details of his results at another place, but has kindly placed his general conclusions at our disposal. From these it appears that in nursing infants the opsonic content is distinctly higher than in the average normal adult, and that dilution is well borne up to 1:100 and in some instances even higher.

| | | |
|---|---|---|
| Baby No. 8 | 1:20 | 84 |
| | 1:40 | 70 |
| | 1:80 | 48 |
| | 1:100 | 22 |
| Baby No. 9 | 1:25 | 80 |
| | 1:50 | 64 |
| | 1:75 | 30 |
| | 1:100 | 20 |

It seems to us that these high values are to a certain extent at least referable to the fact that the nursing infant is practically in a condition of continuous digestion.

But even though a distinct influence upon the opsonic content by the process of digestion is apparent in certain cases this is not manifest in all.

In the case of one of us (S) higher values were thus obtained early in the morning before any food had been taken than two hours after the midday meal. Miss E. similarly showed high values even after fasting absolutely for twenty-one hours, viz.:

1:20 = 92 1:30 = 48

The same individual was placed upon Folin's non-proteid diet for forty-three hours and at the end of this time still showed very active phagocytosis:

1:20 = 88 1:30 = 40

In rabbits also we have found that good feeding *per se* does not increase the opsonic content of the blood beyond the initial values which were noted shortly after the animal had been obtained from dealers. Individual peculiarities exist here, of the causes of which we know nothing as yet.

From a practical standpoint in the study of infections, however, it is well to bear the possible influence of digestion upon the opsonic content in mind. The fact that such a relation may exist throws additional light upon the rationale of "feeding a fever." Moreover, the high figures obtained by us in certain infections thus stand out in even greater contrast, if we bear in mind that in many of these cases the food consumption had been much diminished.

## DISTRIBUTION OF THE OPSONINS IN THE ANIMAL BODY.

In order to ascertain what part, if any, the various organs of the body take in the production of opsonins, rabbits were bled by dividing the femoral vessels and thoroughly milking the body. The organs were then removed and ground up with well washed and dried quartz sand. In the case of the liver it was possible to obtain a sufficient quantity of fluid extract in this manner without the addition of any diluent, but in the case of the other organs it was necessary to use a small amount of normal salt solution. Whenever this was done a large amount of the triturated mush was placed in a tube, digested with the salt solution, and the sand and cellular elements then separated out y centrifugalization. The degree of dilution was in every ase as slight as possible, merely enough to procure a few rops of liquid extract. This was then placed in a separate ube, infected as usual with Staphylococcus citreus, treated with washed human corpuscles, incubated and further examined as described. The results were absolutely negative in the case of striated muscle tissue, liver, spleen, lymph glands, kidneys, intestinal mucous membrane and muscular coats. For the examination of the adrenal glands, brain, pancreas, testicles and ovaries sheep were chosen and for the thymus gland the calf. The results were uniformly negative.

Examination of the bone marrow did not yield entirely satisfactory results. The material was obtained from the bones of a cat, stirred up in a small amount of normal salt solution and incubated at 37° C. for one hour and a half. The cellular material was then removed as far as possible by centrifugalization and the liquid portion examined. It was found that phagocytosis had occurred in 12 per cent. of the washed corpuscles. We fear, however, that the positive result in this case may have been due to the presence of blood serum which could not be removed.

Transudates like blood serum also contain opsonins, but the amount is smaller in the former. With a specimen of fresh hydrocele fluid we thus found only six per cent. of phagocyting leucocytes for a dilution of 1:20 and with a sample of ascitic fluid 10 per cent. with the same dilution. Chyle contains rather more, but also less than the blood serum (60 per cent. in one case in concentrated form).

Our results thus tend to show that the opsonins are essentially components of the blood and the inference naturally suggests itself that they may be formed here. The fact that they are increased so frequently in infections which are associated with hyperleucocytosis would seem to point to the leucocytes as their possible source. Our investigations, however, do not support this view. They show conclusively that no constant parellelism exists between the number of leucocytes and the amount of opsonins. This is well illustrated by Fig. 1, which was obtained in the case of a dog, in which an obstruction of the small intestine had been artificially produced; this experiment is one of a series which one of us (Simon) undertook in association with Dr.

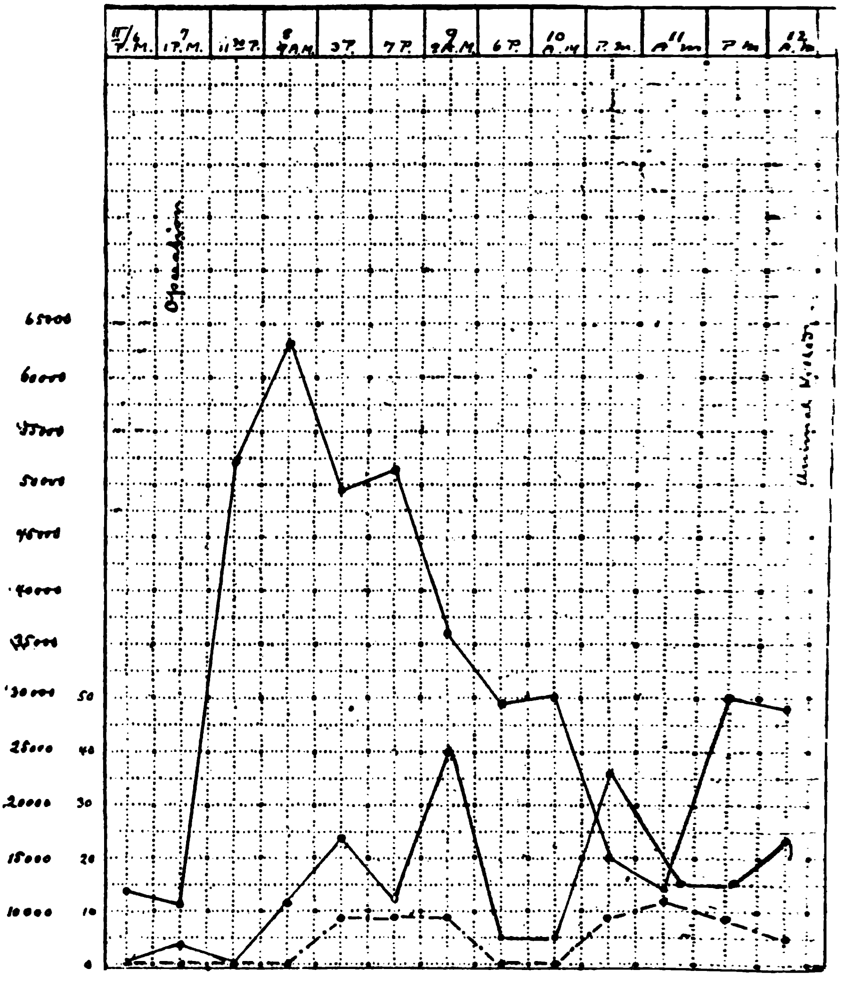

FIG. 1.—Upper solid line=total leucocyte count; lower solid line=opsonins, 1:10 (percentage values); broken line=opsonins, 1:20.

Nelson for the purpose of studying blood changes in intestinal obstruction, a detailed report of which will appear in another place. The animal in this case survived and was subsequently

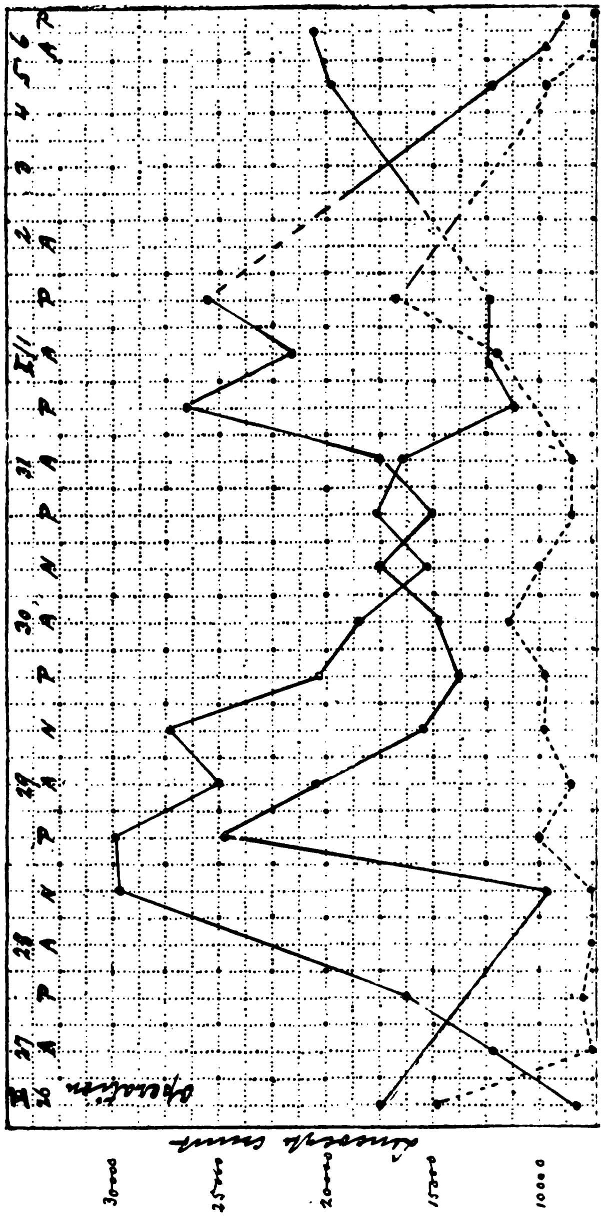

FIG. 2.—Upper solid line=total leucocyte count; lower solid line=opsonins 1:10 (percentage values); broken line=opsonins 1:20.

killed, when it was found that the calibre of the gut had been narrowed sufficiently to allow only the passage of a large sound.

It will be observed that notwithstanding the remarkable increase of the leucocytes, to a point above 60,000, the opsonins both for the 1:10 and the 1:20 dilution gave values which were not at all in excess of what is normal for dogs; for the most part in fact the values were rather lower than those commonly found. The same is seen in Fig. 2, which was obtained from another dog that had undergone a similar operation.

Examination in the case of patients led to the same conclusion. This is well illustrated by the two following observations.

In both cases the symptoms were suggestive of appendicitis. In the one (No. 37) the leucocytes just before operation numbered 23,000; the phagocytosis with a dilution of 1:20 was 18 and negative with 1:30. The appendix in this case was practically normal; there were two patches of induration near the lower end of the ileum and one at the base of the appendix. A definite diagnosis could not be reached; but a history of recent syphilis was considered suggestive. In the second case (No. 68) the leucocytes numbered 23,200—practically the same number as in the first, while the opsonic examination showed 100 per cent. of phagocyting leucocytes even with dilutions of 1:30 and 1:40.

Conversely cases occur where with a normal number of leucocytes the opsonins are very high. This is well shown in the following case:

The patient (No. 137) was a young married woman who was admitted to Dr. Finney's service at the Union Protestant Infirmary with a greatly enlarged spleen and liver. The differential diagnosis rested between Banti's disease and syphilitic cirrhosis. The leucocytes numbered 7500, while phagocytosis with 1:20 was 100 and with 1:30, 96, *i.e.* greatly above the normal.

While the clinical evidence was thus opposed to the assumption of a relationship between leucocytes and opsonins we have made further examinations in this direction, by incubating washed leucocytes for variable lengths of time, suspended in normal salt or in Ringer's solution, and subsequently examining for the occurrence of phagocytosis. The results were invariably negative.

While the above observations render it unlikely that the neutrophilic and amphophilic granulation is concerned in the production of opsonins the rôle of the eosinophiles still remained doubtful. In the case of the incubation experiments just referred to it was conceivable that the small number of eosinophiles in our tubes were not sufficient to bring the opsonins up to that degree of concentration at which phagocytosis would occur. To throw some light upon this question a rabbit was fed about 1750 trichinæ. A marked eosinophilia resulted, which at first was accompanied by a rise in the opsonins. Subsequently, however, they fell and at the last examination no phagocytosis whatever occurred with a dilution of 1:10.

The lymphocytes, according to our negative findings in the case of the lymph glands, can be excluded as opsonin producers.

In the cerebro-spinal fluid from a case of spina bifida we were much surprised to find no evidence of the presence of opsonins.

The examination of fresh seminal fluid and of milk gave negative results.

In exudates the presence of opsonins is variable; but as the element of infection enters into consideration in their production they will be dealt with under a subsequent heading.

## CHEMICAL STUDY OF THE OPSONINS.

Our chemical investigations were rendered particularly difficult owing to the comparative instability of the opsonins; viz. their partial thermolability and the readiness with which various chemical reagents lead to their destruction, or at least inactivation so that they are no longer subject to detection by their specific effect upon the leucocytes. Hektoen and Rüdiger have shown that various salts even in very small amounts are capable of suspending opsonic action when added to the blood serum. They determined this in the case of calcium, barium, strontium and magnesium chloride, potassium sulphate, sodium bicarbonate, trisodium citrate, sodium oxalate, potassium ferro-cyanide and formalin. It was accordingly necessary by means of a series of preliminary experiments to determine what physical processes and what chemical reagents could be utilized without interfering with opsonic action. Then again it was necessary to ascertain, *ceteris paribus*, how long the opsonins will remain active in the blood serum. Wright and Douglas state that the opsonic power of the blood fluids disappears gradually on standing, even when the serum is kept in a sealed capsule, sheltered from light. Under these conditions they found the opsonic power after five or six days to stand at a little more than half of what it was originally. This observation we are able to confirm. With the serum of the pig and of the rabbit we found a vigorous degree of opsonic activity after a week and even longer, no matter whether the serum was kept in the cold or at the temperature of the laboratory. It was interesting to note that with a fair development of bacteria in the serum, we could obtain good phagocytosis after from six to eight days; leucocytes were then found which had taken up the bacteria which had grown in the serum.

We have stated before that Wright and Douglas could demonstrate a partial thermolability for the opsonins, inasmuch as the number of ingested organisms in heated serum was usually less than in unheated serum. But apart from the diphtheria bacillus and Bacillus xerosis, which were taken up as well in the one as in the other, a complete extinction was not as a rule obtained even with those organisms which Wright designates as especially susceptible to opsonic action. In his first experiment with the typhoid bacillus[9] he thus notes that in the heated specimen there was "everywhere considerable phagocytosis" and the phagocytic index is given as 26.2 (circ.) as compared with 24 (circ.) in the unheated serum. In Experiment II with the same organism, on the other hand, the value was 8.1 in the unheated and only 0.8 in the heated one.

Other observers seem to have taken the complete thermolability of the opsonins for granted. But Dean[10] has recently shown that such an assumption is not warrantable and that Wright's results which suggest this possibility are referable to an inherent inaccuracy of the method, viz. the small amount of serum which is used. He could show that normal horse serum, even after being kept for four hours at a temperature of 60° C., still contains a quantity of opsonins sufficient to prepare large numbers of cocci for phagocytosis. Similar results were obtained by Smith[11] in the case of typhoid immune sera. In a recent communication Wright[12] attempts to show, on the other hand, that the phagocytic effect of heated sera is merely referable to opsonins which have escaped destruction and that such sera when diluted with 1.2 per cent. salt solution and subsequently heated are inactive.

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[9] Wright and Douglas, *Proc. Royal Soc.* 1904, lxxiii, 137.

[10] Dean, G., *Centbl. f. Bakt.*, Ref., 1905, xxxvii, 349, 449.

*Behavior on dialysis.*—To study the effect of dialysis upon the opsonins small quantities of blood serum (25–50 c.c.) were placed in sacs of animal membrane or of celloidin and dialyzed against running water for from forty-eight to sixty hours. After this time it was found that the remaining fluid showed no opsonic effect whatsoever, but the appearance of the leucocytes suggested that the negative effect might very well be due to the atonicity of the dialyzed fluid. Upon the addition of a corresponding quantity of Ringer's solution to the dialyzed serum active phagocytosis could again be obtained, although to a less extent, so far as the number of phagocyting leucocytes goes, than with the native serum. This diminution in the amount of opsonins upon first consideration suggests that the substances are to a certain extent dialyzable, but our subsequent experiments have placed an entirely different light upon this apparent loss, so that we believe that we are justified in concluding that the opsonins are not dialyzable. The active phagocytosis which was obtained with dialyzed serum plus Ringer's solution shows that the soluble phosphates and carbonates of the blood are not essential to the process. The fact that the same result also was obtained with dialyzed serum plus normal salt solution further shows that the soluble calcium salts and sulphates of the serum were likewise not of moment. The suggestion that the decalcification of the blood *per se*, by means of the ammonium oxalate or sodium citrate used in washing the leucocytes, was essentially responsible for the non-occurrence of phagocytosis we had previously invalidated by showing that the addition of Ringer's solution to washed corpuscles, without serum, does not restore phagocytic action.

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[11] Smith, R. G., *Proc. Linnean Soc.*, New South Wales, 1905, ii., 269. Wright and Reid, *Biochemisches Centbl.*, Ref., 1906, iv, 125, 126.

[12] Wright and Reid, *Proc. Royal Soc.*, 1906, lxxvii, Series B., 194.

As the addition of normal potassium chloride solution to the dialyzed serum was found to answer the same purpose as the sodium chloride it appears likely that the function of the chloride is in a manner secondary, and merely serves the purpose of maintaining the necessary osmotic tension of the leucocytes. As we have shown, its temporary abstraction during dialysis does not interfere with the subsequent action of the opsonins when the necessary quantity is restored.

While our experiments with dialysis show that the opsonins must be sought among the non-dialyzable components of the blood serum, our experience with the soluble extractives of the various organs suggests that they are specific constituents of the serum. It would hardly seem likely that lecithins, cholesterins, soaps etc. are of moment.

Bearing in mind the close relationship which seems to exist between certain antibodies and the blood albumins experiments in this direction seemed to be indicated, and were rendered possible by our discovery that ammonium sulphate, even when added in substance to saturation, does not destroy the opsonins, although their specific effect can only become manifest again after the excess of salt has been removed by dialysis.

In a series of experiments the albumins of fifty cubic centimeters of serum were thus collectedly precipitated by salting at 40° C.: the precipitate was dissolved in as small a bulk of water as possible and dialyzed against running water for at least forty-eight hours. The contents of the dialyzer were then concentrated in the vacuum to about one half the original volume, diluted with double the amount of 1.2 per cent. salt solution and examined in the usual manner. Active phagocytosis occurred, although the number of phagocyting cells was materially smaller than with the original serum.

The filtrate, after salting out the albumins, as just described, was likewise dialyzed against running water, concentrated in the vacuum to a small volume and treated with salt solution and examined as above. There was no phagocytosis whatever. After having thus demonstrated that the opsonins can be isolated in association with the blood albumins, the albumins were fractioned in the usual manner. The globulin fraction was isolated by half saturation with ammonium sulphate, the precipitate dissolved in a small amount of water and dialyzed. In the water-insoluble portion which separates out (euglobulins) opsonins could then be demonstrated, if a saturated solution in 1.2 per cent. salt solution was prepared and examined in the usual manner, while the pseudoglobulin fraction after concentration in the vacuum, as also the serum albumin fraction, were entirely inactive.

The opsonic content of the euglobulin fraction was less than that of the original serum, but still quite considerable. In one experiment where the examination with the native serum had shown 100 per cent. of phagocyting leucocytes, the concentrated euglobulin solution showed 74 per cent. of the cells still quite active. On diluting, however, to the extent of only 1:10 the result was entirely negative.

These experiments were repeated a number of times with the serum of pigs, calves, sheep, and also with ascitic fluid, and in all cases with the technique described the opsonic effect was obtained with the euglobulin fraction and with this only. The conclusion hence suggested itself that the opsonins, even though themselves not necessarily of the nature of globulins, were nevertheless intimately associated with them as in the case of the various antibodies as shown by Pick.[13] But whereas Pick could demonstrate that diphtheria antitoxin, for example, was quantitatively precipitated from goat's serum in the globulin fraction, the material loss which we met with made us hesitate to regard this conclusion as altogether valid. It seemed not at all impossible that the opsonins, like ferments, were carried down mechanically and in part lost during the physical manipulations (filtration, dialysis), viz. deposited upon the walls of the dialyzer etc. While we have not been able to prove conclusively that this actually occurs, certain experiments which were undertaken to elucidate this point certainly are very suggestive. To this end the quantity of opsonin was first estimated in the native serum. Small tubes were then charged with small quantities of serum together with various substances which in themselves were not thought to exert a destructive effect upon the opsonins, such as coagulated egg albumin which had been dried and finely powdered, wheaten flour, diatomaceous earth, precipitated chalk, filter paper fibre, vegetable charcoal, carmin and platinum black. After having shaken the tubes thoroughly for one half hour the suspended material was thrown down by centrifugalization and the serum examined in concentrated form. The figures representing phagocytosis are given in Table IV.

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[13] Pick, E., *Hofmeister's Beiträge*, 1901, i., 351.

TABLE IV.

| | |
|---|---|
| Native serum | 48 |
| Carmin | 40 |
| Platinum black | 40 |
| Diatomaceous earth | 36 |
| Precipitated chalk | 14 |
| Filter paper | 4 |
| Albumen | 0 |
| Charcoal | 0 |
| Flour | 0 |

These experiments show conclusively that the opsonins can be in part or entirely removed from blood serum by various substances in a manner quite analogous to what we see in the case of various ferments. That they are not destroyed can be shown in the case of carmin and charcoal, for example, by washing the material in 1.2 per cent. salt solution and then exposing it to the action of washed leucocytes, when numerous cells are seen which have taken up the fine granules.

A perfectly analogous result was obtained with cream of milk. Cream was thoroughly shaken with serum for half an hour, separated by centrifugalization and washed with 1.2 per cent. salt solution. With a particle of the resultant butter a fine suspension was prepared in salt solution and incubated for fifteen minutes with washed corpuscles, A drop of this mixture was mixed with a drop of a one per cent. solution of osmic acid and examined, when it was seen that a certain degree of phagocytosis had occurred.

It might of course be argued that the phagocytosis which was thus obtained with carmin, charcoal and fat globules was not dependent upon the absorption of opsonins, but was of the character of spontaneous phagocytosis. This, however, is negatived by the fact that in all experiments a 1.2 per cent. solution of salt was used, which according to Wright eliminates spontaneous phagocytosis. Upon the basis of these experiments it seems not inconceivable that the cause for our finding of the opsonins in the euglobulin fraction of the blood serum may be sought in the fact that these are the only albumins which in the nature of our technique are precipitated on dialysis and would hence carry the opsonins with them. This possibility must be borne in mind in future research. On the other hand our loss of opsonins during our manipulation might be referred to the time element which enters into consideration and would simply represent that portion of opsonin which would become inactive in the native serum upon standing for a corresponding length of time. To enter into this question further did not seem desirable at the present time.

The absorption experiments tend to throw some light upon the nature of the sensitizing of bacteria and the differing behavior of various organisms in reference to the extent of phagocytic action. Similar conditions obtain with various ferments, such as pepsin, which is readily absorbed by animal charcoal, diatomaceous earth, coagulated serum and egg albumin, etc., while bread, flour, lecithin and cholesterin are less active.

### SPECIFICITY OF THE OPSONINS.

The question regarding the specificity of the opsonins is an exceedingly important one. From the standpoint of technique in the investigation of the opsonic content it would simplify matters very much, if it could be shown that the opsonins are not specific, while such a demonstration would also modify present conception regarding the mode of action of bacterial vaccines. As Bulloch[14] states, "this question has hitherto not been touched in the various memoirs," although Wright manifestly assumes that it has been settled in the affirmative. Bulloch comes to the same conclusion, viz. that a high degree of specificity exists. His inference is based upon two classes of experiments. "In the first the opsonic power of a serum was tested against both Staphylococcus albus and the tubercle bacillus. The serum was then mixed with one or other of these microbes and after a sojourn in the incubator the mixture was subjected to the prolonged action of the centrifuge, whereby the microbe was thrown down as a deposit; the supernatant fluid was deprived of its opsonins for the particular microbe with which it had been in contact, while it 'largely' [sic] retains its opsonins for the microbe with which it has not been digested." Unfortunately Bulloch gives no details of his experiments, from which the reader can form an estimate of what is meant by the statement that the serum "largely retains its opsonin for the microbe with which it has not been digested." We must confess that we see no cogent reason why, assuming the high specificity of the opsonins, the opsonin in the experiments mentioned should have been only "largely" retained.

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[14] Bulloch, W., *Lancet*, 1905, ii, 1603.

Our own experiments were conducted in an analogous manner and led to results which are diametrically opposed to Bulloch's conclusion, so far as Staphylococcus citreus, on the one hand, and the typhoid bacillus and colon bacillus on the other are concerned. In the end, as usual, we determined the number of phagocyting leucocytes. In one of these experiments the opsonic power of the native serum (of the pig) was 100 both for the colon bacillus and Staphylococcus citreus. A considerable portion of an agar culture of Staphylococcus citreus was then emulsified in about three cubic centimeters of serum, the mixture stirred for at least fifteen minutes and the organisms thrown down by prolonged centrifugalization. Specimens of the supernatant fluid were then reinfected with staphylococci, on the one hand, and colon bacilli, on the other, and re-examined. It was found that the opsonic power for the staphylococcus had fallen to 20 and for the colon bacillus to 14.

To meet the possible objection that the 20 per cent. for the staphylococcus may have been referable to the presence of sensitized cocci which had not been thrown down by centrifugation the experiment was repeated, but the centrifugalized serum treated with an abundant supply of washed corpuscles and incubated for fifteen minutes so as to remove the sensitized cocci as far as possible by phagocytosis. The corpuscles were then thrown down and the supernatant fluid examined as before; in this case the result was 16 for the staphylococcus and 20 for the colon bacillus.

These experiments were repeated again and again and variously modified. In one instance for example the serum was incubated with a large supply of colon bacilli and kept in the incubator for twenty hours. At the end of that time it was tested as described; six per cent. of the leucocytes showed phagocytosis for the colon bacillus and four per cent. for the staphylococcus. There was accordingly no indication whatsoever of any specificity ✓ of the opsonins; exhaustion for both organisms proceeded *pari passu* in all our experiments.

The same conclusion is suggested by our observation that the opsonic coefficient of extinction is practically the same for different organisms, and irrespective of the initial intensity of phagocytosis, as shown in Table V.

TABLE V.

SHOWING THAT THE OPSONIC COEFFICIENT OF EXTINCTION IS THE SAME FOR DIFFERENT ORGANISMS (PIG SERUM).

| | 1:10 | 1:20 | 1:30 | 1:40 |
|---|---|---|---|---|
| Staphylococcus citreus | 48 | 16 | 4 | 0 |
| Spirillum Metchnikovi | 24 | 20 | 8 | 0 |
| Bacillus coli | 44 | 24 | 16 | 0 |
| Bacillus typhosus | 52 | 24 | 4 | 0 |
| Bacillus Pfeifferi | 22 | 16 | 16 | 0 |
| Bacillus anthracis | 36 | 24 | 8 | 0 |

In a second class of experiments Bulloch reports that the serum of human beings was tested repeatedly both against the tubercle bacillus and against the staphylococcus. He finds that injections of tuberculin produced an increase in the "tubercular" opsonins, while leaving the quantity of "staphylococcus" opsonins unaltered and *vice versa*. It seems to us that experiments of this character are not well suited to settle the question under consideration. In such cases we are essentially dealing with sera in which the entire immunizing mechanism of the body is without doubt thrown into activity and in which other factors besides the opsonins may be affected. We believe Wright and his collaborators have conclusively established the existence of opsonic substances in the blood serum, but we are not prepared at the present status of our knowledge of the subject to admit that no other factors enter into consideration in connection with the process of phagocytosis. It is quite conceivable to our minds that as the result of infection, viz. immunization, substances may be produced either *de novo*, or increased in amount, if pre-existent, which may so influence a given organism that under the subsequent action of a common non-specific opsonin it is taken up in larger or smaller numbers, as the case may be.

This possibility that a second substance besides the opsonins may determine the number of organisms phagocyted, also exists in the case of normal blood serum. It is strongly suggested by the fact that in some individuals a very small number of cocci only is taken up with a dilution of 1:20, for example, so small indeed that we would expect no phagocytosis at all with 1:30 and 1:40, reasoning by what we see in most cases; this dilution may nevertheless be borne without extinction of the phagocytic power. We have repeatedly observed that with a dilution of 1:20 not more than four or five staphylococci are taken up by the phagocyting cells, and that with a dilution of 1:30 we still find two or three and with 1:40 perhaps the same number of organisms. On the other hand we have found that with a fairly large number of cocci pro cell, with a dilution of 1:20, there may be practically no phagocytosis with 1:40. It would seem as though the quantity of opsonins in these cases was fairly uniform, but that there must be another factor which is variable.

This assumption receives a certain amount of support from the following observation:

To eliminate opsonic action blood serum of a pig was diluted one hundred times, infected with cocci and incubated for one half hour. Blood serum from an individual, whose phagocytic value with a 1:20 dilution was 50, was then diluted with the diluted pig serum in the proportion of 1 : 20 and an increase in its phagocytic value to 80 noted. The diluted pig serum *per se* when treated with washed corpuscles showed no phagocytosis and a control specimen with 1.2 per cent. salt solution likewise was negative.

Even more suggestive is the following:

One of our rabbits has shown a remarkably low opsonic content, such that no phagocytosis was ever obtained with a 1 : 20 dilution and only rarely with 1 :10 and then only to a slight degree, not exceeding 8. Pig's serum, which with a dilution of 1 : 20 gave a phagocytic value of 10, was diluted 100 times, infected and incubated for one half hour. The rabbit's serum by itself on this occasion showed 1 :10 = 4 and 1 : 20 = 0, but when diluted 1 : 20 with the diluted pig serum phagocytosis was 47 and the cells contained a fair number of cocci. Pig serum was chosen for these experiments because the phagocytic value for staphylococci is exceptionally great, when used in concentrated form, although it is rapidly lost on dilution.

In order to invalidate the argument that the preceding incubation, previous to the 1 : 20 dilution, might be responsible for the increased phagocytosis salt solution was substituted for the 1 :100 dilution of serum and this incubated with cocci for half an hour. The result showed no difference between the original 1 : 20 dilution of the serum and the subsequent 1 : 20 dilution with the incubated cocci; the phagocytic value in both was exactly alike, viz. 4.

It might further be argued that in some manner the opsonins in the 1 : 100 solution, even though they be insufficient in this dilution to bring about phagocytosis by themselves, become united with the opsonins of the serum, which is about to be diluted 1 : 20, and that the joint effect then becomes manifest. Upon a mathematical basis *per se* this would appear most unlikely; we have shown, however, by experiments of the following nature that this explanation is not valid. To this end the blood serum of one and the same person was used, instead of two different individuals. A 1 : 20 dilution was prepared as usual, as also a 1 : 100 dilution. The latter was incubated for half an hour after infection with cocci and a new dilution of 1 : 20 prepared using the hundredfold dilution in lieu of the salt solution as usual. The result shows conclusively that the 1 : 100 dilution is not capable of raising the phagocytic value of the same individual to a higher level than is obtained primarily. The values in a given case were 38 for the primary 1 : 20 dilution and 36 for the second dilution.

Experiments of this order have incidentally shown the interesting fact that on diluting the blood of one person with that of another, or of one animal with that of another a higher phagocytic value *may* be obtained, but is not necessarily obtained, than in the case of either alone, when diluted with salt solution. This peculiar behavior we are not able to explain at the present time. The problem, however, is reserved for further investigation.

But even admitting the previously mentioned observations of Bulloch and Wright to consideration for the sake of argument, it appears to us that equally cogent reasons can be advanced to prove the non-specificity of the opsonins along similar lines. It is thus difficult to understand why the opsonins for Staphylococcus citreus should be increased in cases in which no evidence of a staphylococcus infection exists whatsoever. In a case of syphilitic cirrhosis of the liver, the phagocytosis for the staphylococcus was thus 100 with a dilution of 1:20 and 96 for 1:30. In a case of extensive miliary carcinomatosis the same values practically were found. The same was found in a case of pernicious anæmia. In the trichinous rabbit similarly there is certainly no ground for belief that an associated staphylococcus infection existed. In a rabbit which had died as a result of a streptococcus infection and in which cultures from the various organs and from the heart blood revealed this organism only, the opsonins for Staphylococcus citreus were enormously increased, viz. at 92 for a dilution of 1:10. In a case of melanotic sarcoma further, in which an extensive excision had been done and in which the opsonic power was quite low, a marked rise, as tested by staphylococci, was observed as the result of Coley's mixed toxin injections, viz. the toxins of Streptococcus erysipelatosus and of Bacillus prodigiosus.

Our findings in cases of appendicitis also may be cited in this connection. Maximal values are here commonly observed and in accordance with the usual bacteriological findings can hardly be referred to a mixed infection of colon bacilli, streptococci and staphylococci.

We have further determined the opsonic content of the blood of tubercular individuals, with our method of dilution, both against Staphylococcus citreus and the tubercle bacillus, and have found the coefficient of extinction the same for both organisms.

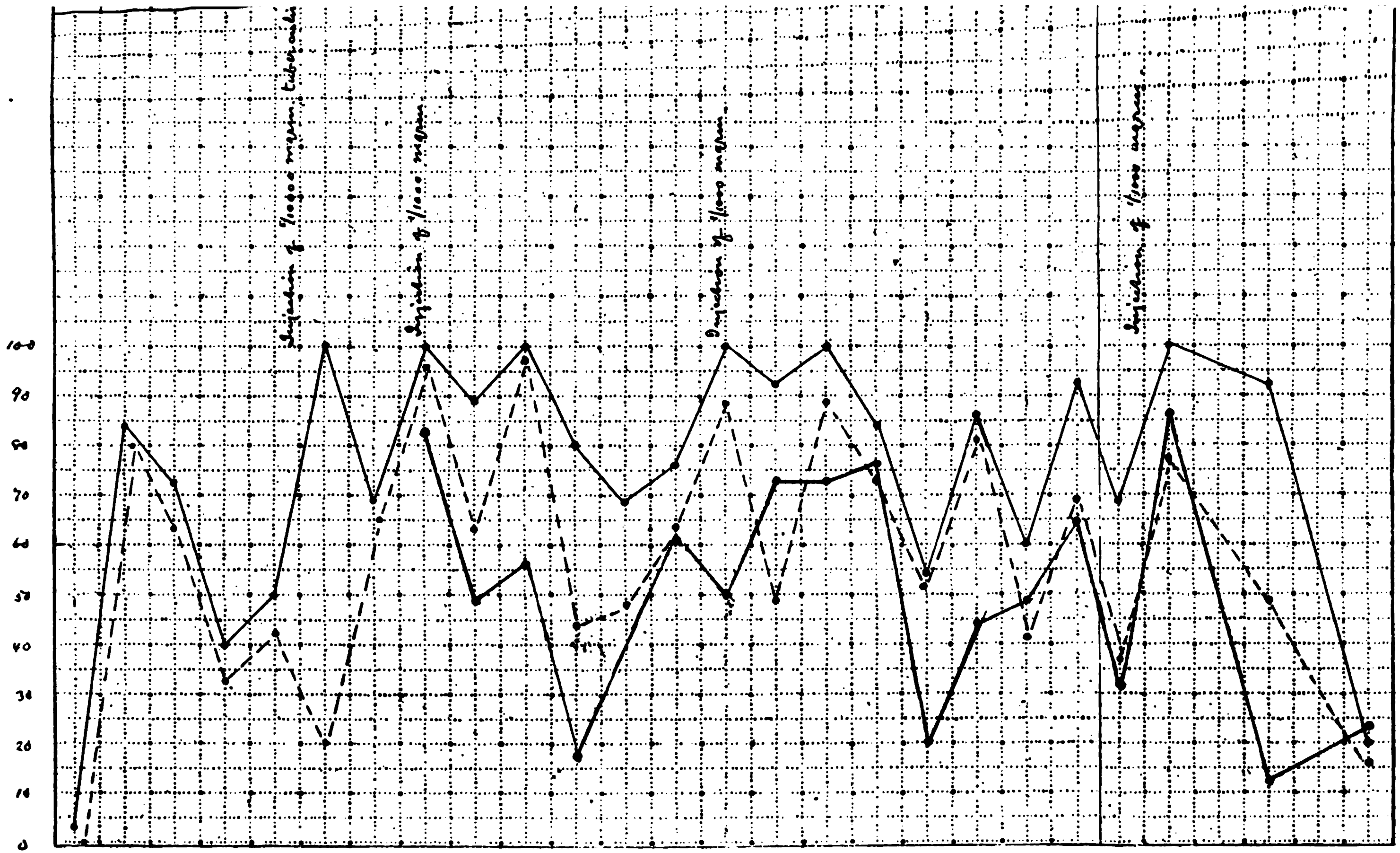

FIG. 3.—Upper solid line represents serum diluted 1:20 (staphylococcus); broken line, a dilution of 1:30 (staphylococcus); lower solid line, 1:20 (tubercle bacillus.)

In one instance of renal tuberculosis for example the value for the staphylococcus with a dilution of 1:20 was 3 and for the tubercle bacillus 2, while with 1:30 there was no phagocytosis with either organism.

Fig. 3, moreover, which illustrates the effect of tuberculin injections upon the opsonic curve, as tested simultaneously with the tubercle bacillus and the staphylococcus, shows that the two run essentially a parallel course and rise and fall together. The minor differences are unquestionably due to the difference between the organisms and their initial variability, corresponding to what we have demonstrated in the case of other organisms (*vide supra*). It is noteworthy also that, although in this case there was no evidence (by culture) of any staphylococcus infection, the staphylococcus curve was nevertheless unusually high and manifestly influenced by the tuberculin injections.

The good phagocytic values which were obtained in cold blooded animals, finally, in which staphylococcus infections hardly occur, would similarly argue against the specificity of the opsonins.

Summing up these various observations, we believe we are justified in concluding that specificity of the opsonins does not exist. Our experiments suggest the possibility that the opsonins may be a constant quantity and that the number of organisms which is taken up by a cell is influenced by a second factor which may be variable.

# THE INFLUENZA BACILLUS IN INFLAMMATIONS OF THE RESPIRATORY TRACT IN INFANTS.*

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The object of the following study is twofold: to determine the presence of influenza-like bacilli in the throat and bronchial mucus of children with and without pulmonary inflammations, and to arrive at a differentiation of these bacilli by means of cultural characters and agglutination reactions.

The material was chosen with a view to including as many different pulmonary conditions as possible. A series of five normal infants, whose history showed no cough since birth, was first examined with negative results. Cases of coryza, bronchitis, broncho-pneumonia, lobar pneumonia, empyæma, rachitis, gastro-intestinal diseases, typhoid fever, diphtheria, tonsillitis, measles, general sepsis following mastoiditis, rheumatism, and cerebro-spinal meningitis, with and without pnuemonia, were next studied. The cases of coryza, bronchitis, and pneumonia were examined within from twenty-four to forty-eight hours after admission, whether the onset had been such as to suggest influenza or not. Only those cases were diagnosed "influenza" which showed the influenza bacillus in the bronchial or nasal secretions. I am indebted to Dr. L. Emmet Holt for the opportunity of studying twenty-two cases of measles at the Foundling Asylum. Of the cases which came to autopsy at the Babies' and Foundling Hospitals blood-agar plates were made from the lungs irrespective of the cause of death.

The method employed was as follows; a sterile swab was rubbed over the posterior pharangeal wall, care being taken to avoid the tongue and the roof of the mouth. In most instances

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this operation causes cough and the raising of some mucus from the bronchi, to which the swab attaches itself. Whenever the mucus was obtained in sufficient quantity, it was washed according to the method of Koch and Kitasato and then spread on blood-agar plates. A similar method was used by Jündell.[1] Rabbit blood was used and mixed with the melted agar at 50° C. When only a very little secretion was obtained, this was spread at once upon the blood-agar plates. The throat cultures were always taken two or two and a half hours after a meal. The plates were examined after seventeen to twenty hours incubation. If no colonies of influenza-like bacilli were found, they were again incubated and re-examined the next day. Only those plates in which the colonies were definitely characteristic and proven by impression preparations to be made up of bacilli having the size, shape, and staining reactions of Bacillus influenzæ, were regarded positive. In the majority of instances subcultures on blood-agar plates served to isolate these bacilli in pure culture, but in some I failed to obtain pure cultivations because of the presence of other organisms, chiefly cocci, in predominant numbers.

CULTURES MADE DURING LIFE.

| | POSITIVE | | NEGATIVE | | Total No. of Infants Examined. |
|---|---|---|---|---|---|
| Broncho-pneumonia | 16 | | 37 | | 53 |
| Lobar-pneumonia | 1 | | 7 | | 8 |
| | With Pneum. | Without Pneum. | With Pneum. | Without Pneum. | Total |
| Normal baby | 0 | 0 | 0 | 5 | 5 |
| Bronchitis | 0 | 11 | 0 | 34 | 45 |
| Empyæma | 3 | 0 | 4 | 0 | 7 |
| Coryza | 0 | 1 | 0 | 2 | 3 |
| Tonsillitis | 0 | 0 | 0 | 3 | 3 |
| Diphtheria | 0 | 0 | 0 | 4 | 4 |
| General sepsis after mastoiditis | 0 | 0 | 0 | 1 | 1 |
| Cerebro-sp. meningitis | 1 | 0 | 2 | 7 | 10 |
| Rheumatism | 0 | 0 | 0 | 1 | 1 |
| Rachitis | 0 | 0 | 0 | 4 | 4 |
| Gastro-intestinal diseases | 0 | 0 | 0 | 22 | 22 |
| Typhoid fever | 0 | 0 | 0 | 3 | 3 |
| Measles | 3 | 5 | 5 | 13 | 26 |
| Tuberculosis | 6 | 2 | 12 | 4 | 24 |

CULTURES MADE AT AUTOPSY.

| | POSITIVE | | NEGATIVE | | TOTAL |
|---|---|---|---|---|---|
| Broncho-pneumonia | 3 | | 10 | | 13 |
| | With Pneum. | Without Pneum. | With Pneum. | Without Pneum. | Total |
| Tuberculosis | 5 | 1 | 0 | 2 | 8 |
| Measles | 1 | 0 | 0 | 0 | 1 |
| Entero-colitis | 0 | 1 | 0 | 3 | 4 |
| General (umbilical) sepsis | 0 | 1 | 0 | 1 | 2 |
| Prematurity (atelectasis) | 0 | 1 | 0 | 2 | 3 |
| Marasmus (bronchitis) | 0 | 1 | 0 | 0 | 1 |

The ages of the children varied from three days to four years, as follows:

| | |
|---|---|
| Under 3 months old | 18 |
| Between 3 and 6 months old | 40 |
| " 6 " 12 " " | 64 |
| " 1 " 2 years " | 80 |
| " 2 " 3 " " | 18 |
| Over 3 years old | 8 |
| Total | 229 |

The positive cases occurred during the months as follows:

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| In | October | of | 30 | cases examined | 10 | were | positive, | equal | 33⅓ % |
| " | November | " | 21 | " " | 2 | " | " | " | 9½ % |
| " | December | " | 19 | " " | 10 | " | " | " | 52 % |
| " | January | " | 41 | " " | 7 | " | " | " | 17 % |
| " | February | " | 36 | " " | 12 | " | " | " | 33⅓ % |
| " | March | " | 32 | " " | 11 | " | " | " | 34 % |
| " | April | " | 48 | " " | 9 | " | " | " | 19 % |
| " | May | " | 14 | " " | 1 | was | " | " | 7 % |
| " | June | | | | 1 | " | " | | |

The table gives a list of the cases studied, with the findings. Under "positive" are recorded the cases yielding influenza-like bacilli; under "negative" are recorded the cases from which the bacilli were not obtained.

Several varieties of organisms grew on the plates, although in many cases (pneumonia, empyæma, tuberculosis) the colonies of influenza bacilli outnumbered the others. The bacteria which were present were: pneumococcus, streptococcus, Staphylococcus albus and aureus, Micrococcus catarrhalis, Diplococcus intracellularis (in a case of cerebro-spinal meningitis on the third day), diphtheria bacillus, pseudo-diphtheria bacillus, an un-identified bacillus which was Gram negative and grew well on all culture media, and finally hemophilous bacilli resembling Bacillus influenzæ. The morphology of the last-named organisms was invariably in agreement with the descriptions of Bacillus influenzæ as given by Pfeiffer. The bacilli were sometimes polar-staining, occasionally forming threads, Gram negative, and growing only upon culture media containing hæmoglobin. When inoculated upon serum-agar, with or without glucose, no surface growth took place, but bacilli could be found in the condensation water, in which they remained alive and transplantable to blood-agar for three days. Sub-cultures from the serum-agar tubes upon fresh tubes of the same medium were unsuccessful.

It would seem from the work of Ghon and v. Preyss [2] that the influenza bacillus grows only in the presence of hæmoglobin. The amount of hæmoglobin required is, however, very small, as was shown in the course of Pfeiffer's [1] work in which the first generation was obtained on serum-agar. Pfeiffer explained the apparent discrepancy by assuming that the sputum used for inoculation contained mere traces of hæmoglobin. Ghon and v. Preyss [2] pointed out that an amount of hæmoglobin too small to be demonstrated with the spectroscope, without the addition of hydrazin, is sufficient to insure the growth of influenza bacilli. Fichtner's [4] cultivations on sputum-agar have not been repeated by others. A small bacillus resembling the influenza bacillus but differing from it by reason of its ability to grow on serum-agar has been obtained by Jochmann and Krause [5] from cases of pertussis. The Koch-Weeks bacillus, which in some respects resembles influenza bacillus morphologically, is easily differentiated in cultures from the latter micro-organism.

In the course of this study several strains of the hemophilous bacillus obtained in cultures were compared. They came from the throat of a nurse (adult) who presented clinical symptoms of uncomplicated influenza; from the throat of an influenzal-pneumonia patient during life; from empyæmic pus; from the lung at autopsy of a case of tuberculosis with pneumonia; from the throat of a patient with measles; from the pus in a case of purulent conjunctivitis; from pertussis sputum.

In smears from the pus or sputum it was impossible to distinguish the several strains by differences in shape and size; but after several generations of growth they varied greatly in size of individual organisms and in the degree to which they formed short threads. Colonies as large as a pinhead, as described by Pfeiffer, were seen in all the strains; inoculations in large amounts on perfectly fresh, moist blood-agar always brought about a return to the small, characteristic colonies. The very large, opaque colonies which Klieneberger[6] described for the hemophilous bacillus isolated by him from the gall bladder in a case of empyæma were not present among our strains, nor did any of our bacilli resemble in size Proteus vulgaris, as did the one noted by Klieneberger, who placed the bacillus isolated by him in the group of pseudo-influenza bacilli.

In a study of the agglutination reactions with the blood of all but two patients negative results were obtained in dilutions higher than 1 to 10. Cases of pertussis are not included in this series. The blood of the adult case of influenza reacted with the homologous bacillus in a dilution of 1 to 20 on the eighth day, and the blood from a case of intermittent influenza (see below) gave a reaction of 1 to 40 during the second and third attacks.

Rabbits were immunized with the strains indicated as "pneumonia," "tuberculosis," "adult influenza," and "eye," until their serum agglutinated the homologous bacillus in dilutions of 1 to 400. The other strains were then agglutinated by such sera in dilutions of 1 to 300 or 1 to 350, showing that the agglutinations were practically common to the group. Absorption experiments confirmed this conclusion. The following table gives the results of some agglutination tests.

Sera of rabbits immunized to influenzal-pneumonia culture:

| | Dilution | 1:10 | 1:20 | 1:100 | 1:200 | 1:400 |
|---|---|---|---|---|---|---|
| "Pneumonia" culture | " | + + | + + | + | + | + |
| "Tuberculosis" | " | + + | + + | + | + | − |
| "Adult influenza" | " | + + | + + | + | + | + |
| "Autopsy pneumonia" | " | + + | + + | + | + | + |
| "Eye" | " | + + | + + | + | + | + |
| "Pertussis" | " | + + | + + | + | + | − |

In repeating my experiments of last year[7] I found that these animals developed agglutinins much more slowly than the old ones had done, and the differentiation of the "pertussis" bacillus from the bacillus of "adult" influenza and "infantile pneumonia" was not so sharply marked as in the previous series. The agglutinins were common to the entire group, the "pertussis" strain, however, showing more frequent and greater variation in agglutination than any of the other strains. The human blood reactions (excluding pertussis) were inactive to all strains, with the exception of the two cases mentioned above, in which the agglutination was too low for purposes of differentiation.

One bacillus which was isolated from a case of pertussis and measles-pneumonia gave reactions as high as those obtained with the "adult influenza," "eye," and "tuberculosis" strains. Whether this difference is to be explained on the ground that there was in this case a possible double infection with "pneumonia" and "pertussis" strains I am unable to say.

The absence of agglutination reactions with the serum from cases of acute pneumonia and bronchitis is to be explained probably by the fact that the agglutinins develop slowly. Rabbits require many inoculations before the agglutinins begin to appear. The blood of all the acute cases of disease was negative, the one positive reaction appearing in the case of intermittent influenza of longer standing. The positive agglutination reaction in pertussis is to be explained by the duration of the infection; and in these cases the reaction does not appear until the end of the second week of the cough.

My study on two occasions of the throats of five normal infants gave me no influenza-like bacilli whatever. Kretz found influenza bacilli in the throats of patients several months after an attack of influenza. He denominates these persons "influenza-carriers."[8] Rosenthal[9] found Pfeiffer's bacillus in the larynx and trachea in about one of six healthy persons, from which he deduced the saprophytic nature of Bacillus influenzæ. No adequate histories are given of the persons studied by him. Wassermann[10] believed that whenever influenza bacilli are found influenza exists, a view which cannot be upheld, unless perhaps in the form given by Ortner,[11] who holds that there cannot be influenza without the presence of influenza bacilli; although the bacilli may occur in the absence of the disease. Davis[12] studied the throats of twenty normal persons and found small numbers of the bacilli in two of them. They had been free from any throat disease for three months, at least, before examination, and remained well for several weeks afterwards. These cases also suffer, in interpretation, from the absence of history of possible contact with persons suffering from influenza.

The cases of broncho-pneumonia and bronchitis had been admitted for these diseases; the stage of onset, therefore, had been passed outside the hospital. The children in whom the influenza bacillus was found were distinguished by showing higher emperature, greater prostration, more illusive pulmonary igns, and longer duration of illness than the children suffering rom broncho-pneumonia uncomplicated by influenza. In the ases of pneumonia and bronchitis which developed in the hosital the bacilli were found on the second day of the disease, before marked pulmonary signs had appeared.

In the three cases of empyæma in which the pus contained the nfluenza bacillus, this organism had been obtained from the bronchial mucus before the operation.

Of the cases of tuberculosis twenty-four were studied, with eight positive results. Six of these gave physical signs of pneumonia. But among the sixteen negative cases, twelve gave similar signs. Eight of these cases came to autopsy: six with and two without positive findings. The question arose as to whether the presence of the bacillus influenced the clinical course of the disease. Of the positive cases five showed pulmonary consolidation, and one had acute miliary tubercles only. The last child showed as the oldest tubercular lesion a small cheesy nodule with a calcareous center in the left pulmonary apex. It seems not improbable that the influenzal infection lighted up this comparatively obsolete lesion.

Ortner [11] observed twelve persons whose sputum contained both tubercle and influenza bacilli, and he remarks that the tuberculosis alone was sufficiently extensive to have caused death. In addition he reports a case of latent tuberculosis which became active and proved rapidly fatal after infection with influenza bacilli.

Ortner [11] and Finkler [13] describe "clinically intermittent influenza." The former means an acute, the latter a chronic influenzal infection of the bronchi. I encountered one such case: B. A., 15 months old, was a rachitic child with a severe anæmia (hæmoglobin 20%); the throat was negative for influenza bacilli. Having been discharged improved, she returned in about four weeks with cough, temperature 103° F., and fine rales over both bases. On the day of admission (Dec. 28) the sputum showed influenza bacilli in almost pure culture. On January 6, the lungs were clear. On January 27, the temperature rose to 103° F., an area of tubular breathing was found, and influenza bacilli were found in large numbers in the throat. On February 3, she was improved. On February 10, a throat culture was negative for influenza. On February 14, the fever and pulmonary signs returned, and cultures were again positive. For the first time the blood gave a positive reaction (1:30) with influenza bacilli. The child had three distinct attacks of acute influenza within three months, two with physical signs of consolidation of the lungs.

Pfeiffer [3] called attention to the persistence of influenza bacilli in the lungs of patients, but thought this especially true when the lungs are the place of diminished resistance before an attack of influenza, as in tuberculosis with cavity formation. Bronchiectasis is in this category, as is chronic bronchitis Ortner [11] states that influenzal bronchitis may exist for months and may run an afebrile course. Lord [14] has also reported cases of long standing, and Boggs [15] observed the bronchiectatic cases. Davis [12] found influenza-like bacilli in the sputum of pertussis patients six months after the attack, and I have been able to cultivate them four months after the onset of the disease.

The influenza bacillus has been found in the nasal secretion in cases of measles by Liebscher [16] in eleven of fifty-seven persons examined during life; and in the lungs at autopsy three times. While 36 per cent. of these influenzal infected cases were fatal, the death rate among non-complicated cases was only 22 per cent. Süsswein [17] found the influenza bacillus in the nose in ten of twenty-one cases of measles and three times in the lungs at autopsy. Jehle [18] found the bacillus in the lungs in eighteen of twenty-three autopsies, and in the tonsils only once. The three writers just quoted consider that this double infection in measles influences unfavorably the clinical course. Jochmann,[19] on the other hand, finding the influenza bacillus in five out of ten autopsies in cases of measles complicated with broncho-pneumonia, believed that its presence has no such effect. Neither Davis [12] nor Klieneberger [6] expressed an opinion on this point. Albrecht and v. Preyss [20] cultivated the organism from the lungs of an infant who died of penumonia three weeks after an attack of measles.

Of the twenty-seven cases of measles which I studied nine gave cultures of influenza bacilli. The number of cases is too small to base any conclusions upon, but it may be remarked that of these nine cases, four terminated fatally, while of the eighteen negative cases seven died.

The presence of the influenza bacillus in the respiratory tract of patients suffering from measles in Europe and the United States at different seasons can hardly be considered as accidental or coincidental. As Ortner [11] has shown, influenza has never entirely disappeared from the continent of Europe since the great pandemic of 1889–90, but has become endemic (at least in the neighborhood of Vienna, from which city he writes), cases occurring every winter and spring. The same reasoning applies to New York City. The catarrhal conditions of the mucous membranes of the eyes and respiratory tract in the early stage of measles must make them favorable situations for the development of the influenza bacillus, which has, at all times, a predilection for these mucous membranes. And since the bacillus is probably always present in cities, in virtue of persistence of cases of chronic influenzal bronchitis and bronchiectasis, and infection of the throats of persons recovered from influenza, the occurrence of influenza baccilli in a large percentage of cases of measles does not seem extraordinary. The frequent bronchopneumonic complication of these cases due often to the influenza bacillus speaks against the view of its saprophytic nature.

In twelve cases of purulent conjunctivitis which I studied I found bacilli belonging to the influenza group. Of these, three were children dying of cerebro-spinal meningitis in which throat cultures proved negative. From these cases a terminal ward infection of the eyes cannot be excluded. Four of the cases yielded the bacilli from the throat, one from the nose and throat, and one (throat culture negative) from the lungs at autopsy. The remaining three cases recovered and yielded negative throat cultures.

That the conjunctiva may possibly be the primary seat of infection with the influenza bacillus was stated by Zur Nedden [21], who found the eye alone involved in five of thirteen persons suffering with conjunctivitis due apparently to the presence of influenza bacilli. A. Knapp [22] found this bacillus in the pus of a severe case of pseudo-membranous conjunctivitis in an infant six weeks old. No other symptoms of influenza existed, but the disease prevailed in New York at the time. Knapp [23] states that there is an influenzal conjunctivitis without other manifestations of influenza. Wynekoop [24] reports a case of very severe pseudo-membranous conjunctivitis from which the influenza bacillus was isolated in almost pure culture, there being mild general symptoms present.

One of the cases in my series is similar to the case reported by Wynekoop. It occurred in a fifteen-months-old child suffering from a pseudo-membranous inflammation of both eyes with much purulent discharge, from which the influenza bacillus was grown in pure culture. It was present also, with other bacteria in the nose. After several days a cough developed; the temperature ranged between 99.6° and 101.4° F., and influenza bacilli were found in the throat. Recovery took place in one week.

In my experience the influenza bacillus was not found in the throats of healthy children nor in the throats of children in whom lesions of the respiratory tract did not exist. When the bacilli occurred in the throat the clinical course of the disease which they complicated was, apparently, influenced by them. This fact seems to be especially true of pulmonary tuberculosis which runs a severer course when complicated by influenzal bacillus infection.

In interpreting the occurrence of influenzal bacilli in the throats of healthy persons, full histories of their previous health and association are necessary. The influenza bacillus persists for long periods after recovery from influenza, and the relapses of the disease may well be ascribed to this condition. Besides which, healthy individuals, themselves insusceptible, may well be the carriers of the bacilli to other and susceptible persons. Hence my experience is opposed, rather, to the view that the influenza bacillus is a harmless saprophyte.

I should also like to point out that the term "pseudo-influenza" bacillus should probably be discarded. The close similarity of cultural characteristics of all the influenza bacilli has been emphasized by Neisser,[25] and my experience with the agglutination reactions leads me to regard all the strains as belonging to one family. The greatest diversity in regard to cultures and agglutination is shown by the influenza-like bacillus obtained from cases of pertussis; but even here I am not convinced that a separate and distinct species can be maintained.

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# THE EFFECTS OF INTRASPINAL INJECTION OF MAGNESIUM SALTS UPON TETANUS.

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In several investigations, some[1] of which have already been published, the writers studied the effects of magnesium salts upon the animal body. The starting point for the investigations was the idea that magnesium salts may have a specific relation to the inhibitory processes of the vital functions. Among the results which have been obtained thus far may be mentioned: (1) deep anæsthesia produced in several species of animals by subcutaneous injections of magnesium salts; (2) the same effect produced with smaller doses of the salts given intravenously; (3) interruption of conductivity by local applications of magnesium salts to nerve trunks; (4) production of anæsthesia by intraspinal injection of magnesium salts. The last effect has a special bearing upon the subject of this paper and hence it will be dwelt upon at greater length.

The first experiments were made upon dogs, cats, and rabbits in which it was found that an injection of a solution of magnesium salts into the lumbar region of the spinal canal caused anæsthesia and paralysis from the pelvis down. The effects were, however, very variable. In a certain number of experiments there was no effect or the animals failed to recover from the paralysis, or succumbed to the injection. The effects became more uniform as soon as monkeys were used for the experiments. On the basis of our experiments on these animals we can state in a general way that a dose of 0.06 to 0.07 gram of magnesium sulphate to one kilo body weight injected through the fourth lumbar intervertebral space definitely produces, sooner or later, anæsthesia and paralysis more or less pronounced, from which the animal recovers in eight to twenty hours. Repeated at proper intervals this dose caused in many of the monkeys the same general effects without recognizable harmful after-results. This dose or one a little larger caused in some instances more pronounced effects; the paralysis spread upward and the animal became, in time, generally anæsthetic. Even from these profound effects the animals recovered completely in the above-mentioned time. A still larger dose produced, in a comparatively short time, a respiratory paralysis from which the animal died without convulsions. But even in these cases artificial respiration through a tracheal tube, begun while the heart-beat was still present, would soon restore life, and if continued long enough the animal would begin again to breathe spontaneously. A dose of the salts which is just sufficient to paralyze the respiration is practically without effect upon the heart-beat and blood pressure, that is, without direct effect. To affect the heart and blood pressure much larger doses are required. This statement may be taken as the rule, from which there are a number of exceptions.

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[1] S. J. Meltzer and John Auer, *Am. Jour. of Phys.*, 1905, xiv, 366.
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S. J. Meltzer and Haubold, *Jour. of Am. Med. Assoc.*, 1906, xlvi, 647.

Using our experience with monkeys as the basis, spinal anæsthesia has already been employed upon human beings. It may be stated here that in human beings a dose of 0.03 gram of magnesium sulphate per kilo produces, after some time, deep anæsthesia accompanied by considerable relaxation of all the muscles, lasting from five to eight hours. The respiration is sometimes reduced, but the pulse and blood pressure remain nearly normal.

In view of the fact that the intraspinal injection of magnesium salts affected profoundly the central nervous system and especially the functions of the spinal cord, it occurred to us that certain pathological states depending upon an abnormal activity of the cord might be influenced by these injections. The first conditions to claim our attention were strychnine poisoning and tetanus. We intend in this communication to report the results obtained in our studies upon experimental tetanus.

To produce tetanus we used tetanus toxin. We had again to contend with the difficulty of selecting proper animals. The animals which have hitherto been employed in tetanus experiments, namely mice or guinea-pigs, were useless for our study, since they could not be relied upon to bring out uniform results. Hence we resorted to monkeys. The cost of monkeys precludes their free use, and furthermore, we did not find in the literature any definite statement of the degree of susceptibility of monkeys to tetanus toxin.

Our studies are, therefore, limited in extent, and we cannot claim to have solved all the problems which the subject suggests. Nevertheless our experiments throw some light upon what we may expect from intraspinal injections of magnesium salts in the treatment of tetanus. One of the questions which seemed fundamental was: Could the intraspinal injections of magnesium salts bring about complete relaxation of the tonically contracted muscles of the tetanic state? Previous experiments had shown us that spinal injections in normal animals produced complete paralysis. But in this case we had only to deal with suppression or interruption of normal impulses, not especially of an inordinate strength. In tetanus, on the other hand, one has not only to deal with a greatly hyperæsthetic cord which, as in strychnine poisoning, causes on slight peripheral stimulation maximal clonic contractions of many muscles, but, unlike any other state, the condition is one of continued contraction of many groups of muscles which are not infrequently in a state of maximum tonicity. Hence we asked ourselves: Will the intraspinal injection be capable of conquering the extraordinary hyperæsthesia of the cord and the exaggerated tonic impulses?

Another question which we asked ourselves was whether the injections would be able to paralyze the muscles implicated in the production of opisthotonus and trismus, in other words, muscles which are innervated by cerebral and cervical nerves, without at the same time reaching the respiratory centre.

To these questions we can now give positive answers. We have seen, more than once, an animal in an exquisite state of tetanus with trismus, opisthotonus, etc., with strong hyperæsthesia and frequent clonic convulsions, lie absolutely relaxed and free from all tetanic symptoms after receiving a single dose of magnesium sulphate (0.08 gram per kilo), while at the same time respiration, heart-beats, etc., were normal; and later the animal recovered completely from the effects of the magnesium salt. Intraspinal injections of magnesium salts are capable, therefore, of abolishing completely, at least temporarily, both tonic and clonic tetanic contractions; and certain doses also affect the muscles of the neck and face without implicating the respiratory functions.

Acting upon an hypothesis which we do not intend to discuss in this paper, we endeavored to ascertain whether these intraspinal injections are capable of retarding the development of the tetanic symptoms. To obtain an answer to this question we had to begin the magnesium injections soon after introducing the tetanus toxin. The discussion of the outcome of these experiments will be deferred until the actual protocols of some of the experiments have been given.

### *Protocols of Experiments.*[2]

Series I. Tetanus toxin 4/100 of a mgr. fatal on the third day for 100 grams of guinea-pig. Hence it equals 1 m.l.d. for guinea-pigs. April 30, 1906, 4 P.M., injected into the right gluteal region of 4 monkeys 2 m.l.d. (8/100 of a mgr.) tetanus toxin for each 100 grams of body weight.

Monkey A, 2828 grams, control. May 2, 12 M. (44 hours after injection), animal very active, possibly slight stiffness of right leg. May 3, 2 P.M. (70 hours after injection), right hind leg definitely stiff, tail deviates to right side, seems weak, not as belligerent as formerly. May 4, 11 A.M. (91 hours after injection), found dead; arms, legs, fingers, etc., flexed and rigid. Was seen alive by the attendant one hour before.

Monkey B, female, 1698 grams. April 30. Soon after the injection of the tetanus toxin 0.4 c.c. of a 25 per cent. magnesium sulphate, = 0.06 per kilo, was injected through the fourth (?) lumbar space. Spinal fluid obtained. No recognizable effect. May 1, 11 A.M. Injected into fourth space 0.45 c.c 25 per cent. magnesium sulphate. Spinal fluid obtained(?). Shortly after hind legs completely paralyzed; 9.55, P.M., completely recovered from paralysis. May 2, animal normal; no injection given. May 3, 2 P. M. (70 hours after toxin injection), right hind leg used less than other, seems somewhat rigid, tail deviates to right. Injected into fourth lumbar space 0.2 c.c. 25 per cent. magnesium sulphate. No marked effect. May 4, 11 A.M. (91 hours after injection of toxin), animal lying on side, right hind leg rigid, left leg less so, jaws closed, cannot be opened passively; arms moderately stiff, but can grasp things. 11.10 A.M., injected 0.25 c.c. magnesium sulphate into fourth (?) lumbar space.

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[2] For the tetanus toxin used in these experiments we are indebted chiefly to Dr. J. J. Kinyoun who supplied us with a dry toxin.

no fluid obtained. 12 M., no definite change, perhaps jaw less locked. 2.10 P.M. injected 0.25 c.c., spinal fluid obtained. 2.15, considerable relaxation of hind legs, almost perfectly flaccid. 4.30 P.M., lies on side, moves arms occasionally, neck relaxed but unable to open jaws. May 5, 10 A.M. (114 hours after toxin injection), very rigid, arms and legs contracted, head retracted. 12.15 P.M., injected 0.25 c.c 25 per cent. magnesium sulphate into fourth space; spinal fluid obtained. 1.05 P.M., hind legs completely relaxed, head only slightly retracted, respiration slow. Jaws still locked. May 6, 1 A.M., found dead by watchman. Animal lived at least 126 hours after injection of tetanus toxin.

Monkey C, 2178 grams. April 30, soon after the injection of the tetanus toxin 0.45 c.c. 25 per cent. magnesium sulphate injected through fourth space; no spinal fluid obtained, no recognizable effect. May 1, 11 A.M., animal normal. Injected through fourth space 0.55 c. c. 25 per cent. magnesium sulphate (0.063 per kilo); spinal fluid obtained. Hind legs paralyzed within a few minutes. 11.20, unable to move, respiration slow, lid reflex active, pupils small. 11.30, respiration getting slower. 11.40, no lid reflex, no respiration, heart-beat cannot be felt. Tracheotomy made and artificial respiration started. 11.50, corneal reflex present again; heart-beat strong again. Artificial respiration continued. 3 P.M., able to breathe spontaneously when artificial respiration is stopped. Artificial respiration continued till 6 P.M., then wound sutured but tracheal canula allowed to remain in place. Animal breathes well. Left without attendance. 9.55 P.M., found dead but still warm.

Monkey D, 1708 grams, female. April 30. Soon after the injection of the toxin 0.4 c.c. of 25 per cent. magnesium sulphate injected through fourth lumbar space; spinal fluid obtained. 20 minutes later animal lying on floor of cage, moves head occasionally; respiration good. May 1, animal normal; no magnesium sulphate given. May 2, animal lively, normal. 12 M., injected 0.3 c.c of 25 per cent. magnesium sulphate into fourth space; spinal fluid obtained. Almost immediately hind legs relaxed. 1.50 P.M., animal lying flat on belly, head resting on floor, breathing very slowly but deeply, moves head slightly but apparently unable to move arms or legs. May 3, normal and fairly active. 2 P.M. (70 hours after injection of toxin), right hind leg moderately stiff, tail deviates to right side. May 4, 11 A.M. (91 hours after injection of toxin), hind legs stiff but not as marked as in "B"; able to open jaws moderately (still able to eat this A.M.). 11.15, injected 0.2 c.c. magnesium sulphate through fourth space; spinal fluid obtained. 11.30, considerable relaxation of hind legs, able to open mouth wide. 3 P.M., hind legs entirely relaxed, grasps with arms. 4.30, hind legs more rigid again, however able to open mouth (eating again) and neck fairly relaxed. May 5, 10 A.M. (114 hours after injection of toxin), lying on side, legs and arms rigid, head retracted. 12.30 P.M., 0.25 c.c. of 25 per cent. magnesium sulphate injected through fourth space; spinal fluid obtained. 1.05, some relaxation of hind legs; arms and head rigid. Injected again 0.15 c.c.; no further change. May 6, 5 A.M., found dead by watchman. Animal "D" lived about 130 hours after the injection of the toxin.

In this series of experiments in which doses of toxin used were double the minimal lethal dose for guinea-pigs, the control animal died in 90 hours and the animals B and D, which received the magnesium, lived at least 125 hours. Furthermore, 44 hours after the injection the control monkey already showed slight stiffness of the right leg, while the treated animals showed nothing; 70 hours after injection the right leg was definitely stiff in the control while in B the right leg "seemed somewhat rigid" and in D the right hind leg was "moderately stiff."

There were also some noteworthy differences between the monkeys B and D. The first injection of magnesium had no affect at all in B, while it nearly completely paralyzed all the extremities in D. The second injection in B, which was larger and given on the following day, only paralyzed the hind legs, while in D the second dose, which was smaller than the first and repeated after forty-four hours, again paralyzed the entire animal. D was apparently more profoundly affected by magnesium than B. Corresponding with this we find that ninety-one hours after the toxin, when the control animal was already dead, the hind legs of D were stiff "but not as marked as in B" and D was able to open the jaws "moderately" while in B "the jaws were closed and could not be opened passively."

We may therefore say that in this series the intraspinal injection of magnesium salts had, apparently, a retarding effect upon the development of the symptoms as well as upon the fatal action of the tetanus toxin, and the retarding action seemed to be the more pronounced the more the magnesium salts had exercised their paralytic influence.

Series II. The same dried toxin used.

A test on guinea-pigs showed the toxin to be unaltered. July 5, injected into three monkeys 6/100 mgr. tetanus toxin for each 100 grams body weight.

Monkey A, 1935 grams; control. July 5, 4.15 P.M., injected the toxin into the right gluteal region intramuscularly. July 6 and 7 no sign of general or local effect. July 8, 11 A.M. (67 hours after injection), right hind leg stiff, "definitely stiffer than in the other monkeys"; right arm seems stiff; almost unable to open jaws. July 9, 10.30 A.M. (about 90 hours after injection), found dead; "still warm." Head retracted, jaws locked, arms flexed at elbows and wrists, pressed on body.

Monkey B, male, 2000 grams. July 5, 3.15, received toxin into right glutei. 3.25, injected through third space (?) 0.6 c.c. magnesium sulphate; no spinal fluid obtained; no effect. July 6, 2.25 P.M., injected 0.6 c.c. magnesium sulphate through third space; spinal fluid obtained. Within two minutes hind legs completely paralyzed, flaccid, no sensation; within ten minutes front legs weak, falls on side. 3.20 P.M., lid reflex active, pupils small, entire body completely relaxed.

July 7, active, no magnesium given. July 8, 11 A.M. (68 hours after the toxin), right hind leg stiff, "less than control," opens jaws wide, eats, tail slightly deviated to right. 11.20, injected through third space 0.4 c.c. of 25 per cent. magnesium sulphate. 12 M., no distinct paralysis, but lies quietly in cage. Injected again 0.2 c.c. magnesium; spinal fluid obtained. 1 P.M., very quiet, feels no pinch in hind legs but uses them to grasp. July 9, 10.30 A.M., (92 hours after toxin), head retracted, jaws moderately locked, able to open about 0.5 cm.; legs and arms stiff, unable to sit or stand. 10.45, injected 0.5 c.c. 25 per cent. magnesium sulphate between fourth and fifth lumbar vertebræ; no spinal fluid obtained. 11.40, no definite effect. Injected 0.3 c.c. magnesium sulphate between third and fourth lumbar vertebræ; spinal fluid obtained. 12.45, no marked relaxation. Injected 0.3 c.c. magnesium sulphate between third and fourth lumbar vertebræ. 1.10, legs and abdomen much relaxed, respiration slow. 2 P.M. hind legs completely relaxed, arms not so stiff, head moderately retracted. 5 P.M., hind legs flaccid, front legs a bit stiffer now, head more retracted, jaws cannot be opened passively. July 10, animal died at 10.40, A.M., 114 hours after injection of toxin.

Monkey C, female, 2540 grams. July 5, 3.45 P.M., received toxin into right glutei. Injected 0.7 c.c. 25 per cent. magnesium sulphate between third and fourth lumbar vertebræ; spinal fluid obtained. No noticeable effect, later shows marked weakness in hind legs. July 6, 2.30 P.M., monkey normal. Injected between third and fourth lumbar vertebræ 0.6 c.c. 25 per cent. magnesium sulphate; spinal fluid obtained. Hind legs paralyzed almost at once, front legs show weakness, head sinks to floor. Placed inclined, head higher in cage. 4 P.M., upper part of body more relaxed than before; lid reflex active. July 7, very quiet, moderate weakness, eats. No magnesium given. July 8, 11 A.M. (68 hours after toxin), right leg moderately stiff, less than control, eats, opens jaws wide, yawns. 12 M., injected 0.4 c.c. 25 per cent. magnesium sulphate between third and fourth lumbar vertebræ; spinal fluid obtained; shortly after hind legs paralyzed, right hind leg apparently less stiff than before; lies in cage. July 9, 11 A.M. (92 hours after toxin), able to support herself; ate this afternoon; no retraction of head; able to open jaws moderately; front legs held flexed, close to body, but are moved freely; grasps objects. Hind legs held flexed at hip and knees, moderate resistance to extension. Tail practically straight. Animal shaken by spasmodic contractions. 11.20 A.M., injected 0.7 c.c. 25 per cent. magnesium sulphate between third and fourth lumbar vertebræ; spinal fluid obtained. 11.25, no spasmodic movements so far. Suddenly respiration stopped, time lost with compression of chest, traction of tongue, etc. Later tracheotomy made and regular artificial respiration begun. No effect, animal dead.

July 9, a fourth monkey was injected with 1½ m.l.d. of the toxin used on July 5. Toxin tested on guinea-pigs and proved to have the same strength as on July 5.

Monkey D, female, 2362 grams. July 9, 12.20, P.M. received in the right glutei 1.5 m.l.d. of toxin per 100 grams. 12.26, injected 0.5 c.c. magnesium sulphate between third and fourth lumbar vertebræ; spinal fluid obtained. Some magnesium lost. 1.05, no visible effect. Injected again 0.3 c.c. magnesium sulphate; spinal fluid obtained. 2 P.M., hardly able to use legs, scratches buttocks frequently. 5 P.M., same, except that she does not scratch; does not feel pressure of foot on tail. July 10, uses legs well. 11.30, injected 0.4 c.c. 25 per cent. magnesium sulphate between fourth and fifth lumbar vertebræ; spinal fluid obtained. In a few minutes unable to use hind legs, scratches buttocks again. 5 P.M., able to sit up but cannot use hind legs. July 11, 2.30 P.M., no sign of stiffness or paralysis. Injected 0.3 c.c. 25 per cent. magnesium sulphate between fifth lumbar and sacrum; no fluid obtained. 3 P.M., unable to use hind legs, otherwise active. 5 P.M., hind legs completely paralyzed, scratches lower parts. July 12, still scratches herself; uses hind legs; right hind leg a little stiff (11 A.M., 71 hours after toxin). 12 M., injected 0.4 c.c. 25 per cent. magnesium sulphate between fourth and fifth lumbar; spinal fluid obtained. 1.30 P.M., hind legs paralyzed and relaxed; scratches herself continually. July 13, 11 A.M. (95 hours after toxin). Right hind leg definitely stiff but can grasp with toes. Able to open jaws but not as widely as yesterday. No scratching. Injected 0.4 c.c. 25 per cent. magnesium sulphate between fourth and fifth lumbar vertebræ; spinal fluid obtained. 11.20, rests head on table, no scratching. 4.15, hind legs flaccid, uses arms freely; scratches lower parts, occasionally even bites skin. 4.20, injected 0.25 c.c. between fourth and fifth lumbar vertebræ; spinal fluid obtained. No notes on effect. July 14, 11 A.M. (119 hours after toxin), lies on left side curled up, legs flexed on hips and knees, body curved, opisthotonus, head moderately retracted, can be moved, able to protrude tongue through jaws; unable to eat. 10.40, injected 0.5 c.c. magnesium sulphate between fourth and fifth lumbar vertebræ; spinal fluid obtained. 11.45, hind legs fully relaxed, flaccid. 12 M., no pain felt in lower two-thirds of body. 1.30, lies on inclined board, head down; looks greatly relieved and improved. July 15, animal died about 6 A.M.—about 138 hours after injection of toxin.

In Series II we again see the control animal dying 90 hours after the injection of toxin, while the animals which received magnesium lived, in case of B 114 hours, and D 138 hours, while C lived only 92 hours. The last animal, however, died not of tetanus but of an overdose of magnesium while she was still in a good condition.

There was, therefore, marked difference in the development of the symptoms between the control and the treated animals. Sixty-seven hours after the injection of the toxin the control was unable to open the jaws, the forearms seemed stiff, and "the right hind leg was definitely stiffer than in the other monkeys." The other three monkeys at this time opened their jaws wide, were able to eat without difficulty, and were using their legs with only little impediment.

To recapitulate briefly, the series of eight monkeys received tetanus toxin in quantities apparently above the minimum lethal dose. Two served as controls and six were treated with magnesium sulphate intraspinal injection in varying doses and at varying intervals. Two of the latter animals died from overdoses of the magnesium. The two controls died about ninety hours after the injection of the toxin, while four animals which received smaller doses of the magnesium survived the controls, one by twenty-four hours, one by thirty-six hours, one by forty hours, and one by forty-eight hours. In five of the magnesium treated animals the onset and the development of the tetanic symptoms were distinctly retarded in comparison with the controls. Furthermore, the retardation appeared to be more pronounced in those animals in which the injection of magnesium produced the most marked effects.

The completely relaxing effect of the injections of magnesium salts was evident in some of the injections given in these series. For reasons which will be mentioned presently we had to avoid larger doses in these series just at the advanced stage of the tetanic symptoms, and we were, therefore, frequently not in a position to demonstrate, by a single dose of the magnesium salts, the complete overpowering of all tetanic symptoms, especially at their height. But we were able to do this, as stated above, in other experiments in which magnesium was not given from the very beginning and in which the interval between injections was sufficiently long to permit the use of large doses. Here in these cases a single injection of a sufficient dose would cause in a short time absolute relaxation, and suppress completely a condition of violent tetanus.

Repetition of the injections, in the above series, brought to light an instructive fact with regard to the effects of intraspinal injections which compelled us to avoid in serial injections the use of larger doses. While a single dose of 0.06 or 0.07 gram of the salt per kilo animal would in normal monkeys produce hardly more than paralysis of the posterior extremities and pelvic region, and never proved fatal, we saw that in animal C of Series I on the second day a dose of 0.063 gram per kilo abolished the respiration within a short time after the injection; and in animal C of Series II a dose of 0.069 gram per kilo administered on the fifth day killed the animal within a few minutes, while the same dose injected on the first day had only a very moderate effect. The fatal result was, evidently, caused by cumulative action of the magnesium salt. Some of the injected solution, apparently, remains for a time in the spinal canal or in some part of the central nervous system. At present it cannot be stated how much of the solution and how long it remains there. As the tetanus intoxication progressed we had to grope our way carefully with regard to the dose of magnesium salt to be injected as well as with regard to the intervals which had to pass between the injections. Towards the last, therefore, the doses given were often small and inefficient as we did not care to risk losing the animal through an overdose at this stage of the disease.

It is obvious that in judging the significance of our results the last mentioned fact has to be taken into account. Had we been less bound by economical considerations, and had we risked towards the end of the disease somewhat larger doses at more frequent intervals, we feel confident that we would have been successful in some cases in prolonging the life of the animal by many more days, although we might have killed some monkeys prematurely by an overdose of magnesium. With regard to Monkey D in Series II, for instance, we feel confident that had we given a slightly larger dose on the seventh day and repeated the dose of magnesium also on the evening of that day, the animal would not have died on the eighth day following the injection of toxin. Furthermore, it is possible that in some cases we might even have been successful in tiding over the animal until the newly formed antitoxin could have overtaken the balance of free toxin, and the metabolic processes of the body have mastered the toxin fixed by the nerve cells; or in other words we might have saved the animal's life.

While, therefore, as far as this short series of experiments is concerned we were unsuccessful in saving, by means of intraspinal injections of magnesium, the lives of any of the animals poisoned with tetanus toxin, the results do not justify the conclusion that such intraspinal injections are incapable of saving tetanic animals. On the contrary, the experiments brought out certain rather encouraging facts. It was established that the intraspinal injections are capable of abolishing completely all tonic and clonic muscular contractions, thus eliminating for many hours at a time one at least of the most dangerous factors in the disease. Moreover, the experiments disclosed the important fact that intraspinal injections of magnesium salts are capable of retarding perceptibly the progress of the disease.

The last fact is a new and, in a certain sense, an unexpected point, inasmuch as the retardation of the progress and development of the tetanic symptoms cannot be considered simply as a consequence of the inhibitory property of the salts or of their ability to relieve the symptoms. This effect was, however, not wholly unexpected by us, since, as we stated above, on the basis of a certain hypothesis which we had formed, we looked for it, and, therefore, in a part of the experiments we began to inject the salts long before there appeared tetanic symptoms to relieve. As regards the hypothesis itself, we believe it would be premature to state it here; we intend, however, to discuss it shortly in connection with another investigation.

We mean to mention in this place some distinctions with reference to our problem between human beings and monkeys which might have theoretical as well as practical significance. There is in the first place a difference in the two species of extension of the spinal cord in the lumbar canal. The autopsies on monkeys taught us that in the species which we employed the cord extends to the sacrum. Any injection made, therefore, in the lumbar region could produce injury of the cord. It is, however, noteworthy that every monkey except one (an animal which had been used previously for other experiments) showed, nevertheless, no effect due to this cause, although in some cases repeated injections were made. Yet it cannot be excluded that in some cases a part of the fluid may have entered and remained in the cord, a possibility which might perhaps account for variation of effect where an identical dose of the salt was employed under apparently the same circumstances. In human beings, on the other hand, the injections are always made below the cord.

Another point of distinction arises from the circumstance that in monkeys only very little spinal fluid can be obtained and none of the injected fluid could be recovered, even soon, after the injection. When intraspinal injections are made in human beings, spinal fluid equal in amount to the injected quantity of the salt solution can be removed previously. This preliminary lowering of the pressure within the canal aids possibly in a more rapid distribution of the solution through the entire canal, and is perhaps partly responsible for the observed fact that in human beings the effective dose of the magnesium salt (per kilo body weight) is not even one-half that which is required for monkeys. Furthermore, in human beings "washing" of the canal proved to be a helpful procedure. When the respiration became too slow spinal fluid was withdrawn and saline was injected several times and again withdrawn. The patient soon recovered from the effects of the magnesium salts. In monkeys we were deprived of such a help in case of danger and had, therefore, to keep away from the danger line.

Intraspinal injections of magnesium sulphate have already been employed in a small number of cases of human tetanus. We shall append here brief notes, as far as they have come to our notice, concerning these cases.

Case 1, described by Dr. J. A. Blake.[1]—A boy of fifteen had trismus, opisthotonus, etc , and a temperature of 104.4 F. This was on the sixth day after the first tetanic symptoms appeared, which began seven days after injury to the fingers, and he had already received 115 c.c. of tetanus antitoxin and morphine without relief. The boy's weight was about 65 kilos and 4.5 c.c. of a 25 per cent. solution of magnesium sulphate was injected into the spinal canal, that is 0.02 gram per kilo. "Six hours after the injection the jaws could be opened wide; the stiffness of the neck and back had disappeared . . . and the temperature had fallen to 102.6 F, the pulse to 104; the respirations were 14." Thirty-three hours after the injection of magnesium sulphate its effect seemed to have entirely passed off and another injection of the same amount was made. "In two and a half hours he was relaxed and asleep." After twenty-four hours there was a steady increase in the spasmodic contractions and after thirty-seven hours the opisthotonus returned. Another injection was then given of 8 c.c. of 12½ per cent. magnesium sulphate. This injection was again followed by complete relaxation. A similar injection to the last was repeated after twenty-nine hours with a similar result. In the next four days "the symptoms were not severe and the patient was kept comfortable with morphine and chloral." On the sixth day after the last injection "the opisthotonus became more severe and there was considerable rigidity of the jaw muscles and considerable pain," so that a fifth injection of magnesium was given which was followed by marked relief. After this the convulsions were much less frequent and severe, and gradually disappeared, so that after fourteen days the boy was practically well.

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[1] J. A. Blake, *Surgery, Gynecology, and Obstetrics*, 1906, v, 541.

In this case with complete recovery five intraspinal injections were given without any injurious effect whatsoever. Each injection produced complete relaxation lasting quite a long time. There was no evidence of a cumulative effect, but, on the contrary, "a certain amount of tolerance seemed to be established for the sensory impulses and the cerebrum . . . the motor symptoms were practically the same after each injection." No rise of fever occurred after the last three injections.

Case 2, boy reported by Dr. Samuel Logan,[4] New Orleans.—Tetanus set in on the ninth day after an injury of the hand inflicted by a toy pistol. On the third day of the disease, after antitoxin and chloral and bromide had been given in full doses with no avail, 4 c.c. of a 25 per cent. solution of magnesium sulphate were injected into the spinal canal—1 c.c. for every 20 pounds of body weight—under anæsthesia. It was noticed "that under complete anæsthesia the abdominal muscles were never relaxed, the abdomen being rigid and hard as a board on palpation." Forty minutes after the injection all the muscles of the body were completely relaxed, the abdomen was soft, and the mouth could be opened readily; the patient was perfectly conscious and said that he felt well. About five hours after injection a profuse bronchorrhœa developed and the breathing grew rapid and shallow. Atropin seemed to have relieved this condition perceptibly. Pulse was 140 and thready. (On the day before the injection the pulse varied between 120 and 140 and the respiration between 24 and 36.) About twelve hours after the injection the tetanic symptoms began to come back, and eleven hours later the muscles of head, neck, and abdomen were rigidly contracted, but the general convulsions did not return. Temperature 102 F., pulse 150 and thready; respiration 30. Under anæsthesia a small dose of magnesium sulphate (50 minims of a 25 per cent. solution) was again injected. Thirty-five minutes after the injection, all the muscles of the body were again completely relaxed. Three hours after injection bronchorrhœa again developed which was again partially relieved by atropin and applications of heat. The general condition of the patient, however, gradually became worse; the pulse rose to 192, the respirations to 50, and the patient died in a state of complete relaxation about sixteen hours after the last injection. No autopsy was made.

In this case too the intraspinal injections of the magnesium abolished promptly and completely all tetanic symptoms. The bronchorrhœa was probably no after effect of the magnesium injection, but possibly of some complication (pulmonary tetanus?); the high pulse and respiration before the injection of magnesium would seem to indicate this.

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[4] Samuel Logan, *Journal Am. Med. Assoc.*, 1906, xlvi, 1502.

Case 3, observed by Dr. Charles D. Peck, at the French Hospital. New York. We are enabled, by the courtesy of Dr. Peck, to give the following notes.—A man thirty-five years old received an insignificant wound in the sole of the foot, which healed rapidly. Four days after the injury a violent tetanus broke out. He received large doses of antitoxin and sedatives but no relief. An intraspinal injection of magnesium sulphate, 1 c.c. of a 25 per cent. solution to every 25 pounds of body weight, had only a moderately relaxing effect. After twenty-four hours the injection was repeated but this time 1 c.c. of the solution was given for every 18 pounds of body weight. The anæsthetizing and relaxing effect was now complete. We had the privilege of seeing the patient about four hours after the second injection was made. He was under perfect general anæsthesia; all parts of the body were completely relaxed, the abdominal muscles being especially soft; no sign of tremor or twitch anywhere except now and then a slight retraction of the right corner of the mouth produced by tickling the nasal mucous membrane. Lid reflex was present, respirations 22 per minute, fairly deep. Pulse 120, of fair quality, and temperature 102 F. The temperature and pulse were the same as before the injection. Three hours later the patient was given an intravenous injection of 60 c.c. antitoxin. The patient died suddenly about two hours later. Dr. Peck informs us that the respiration remained good to the end.

These three cases show that in human beings the intraspinal injection of magnesium sulphate is capable of abolishing completely for quite long periods the severest symptoms of tetanus. In the first case it was apparently the means of saving the patient's life. That the other two patients died while their muscles were in a perfectly relaxed condition may be taken to indicate that tetanus toxin has other poisonous effects besides those which produce the tetanic symptoms. Since, however, other interpretations are possible, we cannot afford to enter here into a discussion of this subject.

## CONCLUSIONS.

Intraspinal injections of magnesium sulphate, in doses which do not affect the respiratory centre or other vital function, are capable of abolishing completely all clonic convulsions and tonic contractions in cases of human tetanus, and experimental tetanus in monkeys. The relaxing effects of the injections may last twenty-four hours or longer.

In experimental tetanus in monkeys early intraspinal injections of magnesium salts are capable of retarding the progress and development of the tetanic symptoms.

# THE OCCURRENCE OF SPECIFIC IMMUNITY PRINCIPLES IN THE BLOOD OF VACCINATED CALVES.*

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The immunity which human beings develop towards vaccinia, after a successful vaccination, is the classical example of an artificially induced active immunity. The duration of the immunity to vaccinia is variable but extends frequently over many years. Many animals are subject to inoculation with vaccinia and they show, as in the case of man, a state of immunity following the disappearance of the successful vaccination. The calf, as is well known, is now generally employed for the production of the vaccine-virus which is used to protect human beings from smallpox. At one period in the development of specific serum therapy an attempt was made to utilize the immune principle which it was presumed existed in the blood of animals which have passed through a successful vaccination. The hope was entertained that, possibly, the serum of the vaccinated calf might exercise curative properties upon persons suffering with smallpox. This hope has not been realized. In order to prove the existence of immunity principles in the blood of vaccinated animals the attempt was made to protect, by inoculation of the blood of these calves and monkeys, fresh animals from vaccinia. In spite of some successful results the experiment may be said to have often failed.[1]

The failure of the blood of vaccinated animals regularly to confer passive immunity or to exert curative effects cannot be held to disprove the existence in the blood of immunity substances. Tests of the kind mentioned are exceedingly gross. Moreover, in the case of vaccinia and variola we labor, at present, under the great disadvantage of being ignorant of the nature of the specific microorganisms of the diseases. Once in our hands it is probable that these microorganisms would quickly yield evidences of antibody or immunity-principle formation. Hence the question arises whether by any other method than has thus far been employed the existence of antibodies can be shown for vaccinia and for smallpox. Should it be possible to demonstrate, by an accurately quantitative method, the existence of antibodies in one of these diseases it would not only be safe to assume the existence of antibodies in the other, but the way would be opened to ascertain whether the immunity principles of vaccinia and smallpox are qualitatively identical. I have succeeded, by the application of the Bordet-Gengou method of fixing hæmolytic complement, in proving the existence of antibodies in the blood serum of vaccinated calves; and I am engaged upon the study of smallpox with the same method. Should this second investigation yield positive results a new diagnostic measure for this disease would be available.

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* The work reported here was begun in the Pathological Laboratory of the Johns Hopkins University and completed in the Rockefeller Institute for Medical Research.

[1] The literature covering this subject is reviewed by Sternberg and Reed, *Trans. Amer. Assoc. Phys.*, 1895, p. 57 and by Copeman, Vaccination, London, 1899.

As long ago as 1901 Bordet and Gengou [2] showed that if fresh guinea-pig complement was added to typhoid, cholera, plague, or anthrax bacilli which had been treated previously with their specific anti-sera, the complement would be bound or absorbed by the bacilli. The test of the union of complement and bacilli was made with sensitized rabbits' erythrocytes, which remained intact under these conditions, but underwent hæmolysis in the control experiments in which the anti-sera were omitted. By means of this method the existence of antibodies was demonstrated in the serum of a horse immunized to plague bacilli, and in the blood of two persons who had recently recovered from typhoid fever. Malvoz [3] in the same year employed this method to show the presence of antibodies in the blood of animals inoculated with cultures of yeast. The later work along this line, through which antibodies for several microorganisms in animals, and in the blood of persons suffering from various diseases, have been demonstrated, dates from Moreschi's [4] experiments on the combination of complements and specific immune sera and Neisser and Sachs' [5] application of the principles of these methods to the detection of traces of blood in medico-legal practice.

The credit of using practically the method of complement absorption for the detection of antibodies in infectious diseases must be given to Wassermann and his pupil Bruck,[6] since in the hands of Bordet and Gengou no general application was made of their discovery. But the revival of the method followed from Moreschi's and Neisser and Sachs' experiments. Wassermann and Bruck[7] employed it to demonstrate, as they thought, the existence of tuberculin in tuberculous foci, and Wassermann, Neisser and Bruck[8] and Detre[9] believed that by its means they discovered the existence of antibodies in syphilis. Kolle and Wassermann[10] have applied the method successfully to the estimation of the quantity of immune bodies which exist in the anti-serum prepared in the horse for the diplococcus of epidemic meningitis.

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[2] Bordet and Gengou, *Annales de l'Institut Pasteur*, 1901, xv, 289.

[3] Malvoz, *Annales de la Soc. Med.-Chir. de Liege*, 1901, xl, 275.

[4] Moreschi, *Berliner klin. Woch.*, 1905, xlii, 1181.

[5] Neisser and Sachs, *Berliner klin. Woch.*, 1905, xlii, 1388.

On account of the similarity of methods used for the study of syphilis by Wassermann, Neisser, and Bruck, and vaccinia by myself, I will give briefly the method employed by the former, prefacing what I say by the statement that my experiments were well advanced when their paper on syphilis appeared. Although the specific organism of syphilis has now probably been discovered, yet so long as it cannot be cultivated and obtained in mass, resort must be had to pathological products which contain the microorganism in numbers large enough to yield a specific extract of them, or an extract, at least, containing the specific products of their growth and metabolic activities. They employed for extraction condylomata and the organs of a still-born syphilitic child; and for the immune bodies they used the inactivated blood serum from cases of syphilis. Wassermann, Neisser, and Bruck found that when these extracts were combined with the inactivated syphilitic serum and fresh guinea-pigs' serum, the mixture having been placed in the incubator for one hour, that the subsequent addition of sensitized corpuscles, which in the control tubes underwent hæmolysis, were in the other tubes still intact at the end of twenty-four hours.

For the purposes of the studies on vaccinia the vaccine virus and serum of vaccinated calves were obtained through the courtesy of the H. K. Mulford and Parke and Davis companies, to whom I wish to express my indebtedness. I wish to thank, especially, Dr. J. J. Kinyoun of the Mulford Company for repeated acts of kindness. The normal sera of calves and oxen were obtained directly from slaughter-houses. The inactivation of all the sera was conducted in a water-bath, at 56° C., this temperature being maintained for one half-hour. The virus was ground finely and suspended in 0.85 per cent. salt solution. The preservation was made with 0.4 per cent. carbolic acid or with toluol. The emulsions thus obtained were shaken for three or four days in the mechanical shaker and then subjected to centrifugalization until clear, the fluid portion being decanted from the sediment. This extract was used in the experiments.

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[6] Wassermann and Bruck, *Med. Klinik*, 1905, i, 1409.

[7] Wassermann and Bruck, *Deutsche med. Woch.*, 1906, xxxii, 449.

[8] Wassermann, Neisser, and Bruck, *Deutsche med. Woch.*, 1906, xxxii, 745.

[9] Detre, *Wiener klinische Woch.*, 1906, xix, 619.

[10] Kolle and Wassermann, *Deutsche med. Woch.*, 1906, xxxii, 609.

Each sample of extract was tested (1) for hæmolytic action on blood corpuscles, and (2) for its absorbing power for complement. All the extracts when used in relatively large amounts caused hæmolysis, an action which is to be attributed, probably, to the effects of tissue-products described by Metschnikoff[11] and Korschun and Morgenroth.[12] As a rule one cubic centimeter of the vaccine-extracts produced in twenty-four hours complete hæmolysis of one cubic centimeter of 5 per cent. washed hens' corpuscles, and 0.8 cubic centimeter of the extracts produced partial hæmolysis. The extracts when used in large quantities also combined or neutralized the action of complement.

An amount of the extract as large as 0.6 cubic centimeter would, if permitted to act for one hour at 37° C., annul the hæmolysis of washed corpuscles and inactivated serum added subsequently. Hence in every experiment the action of the extract, either in producing hæmolysis or in annulling it by fixation of complement, was carefully excluded through the employment of sufficient controls.

The hæmolytic serum was obtained by inoculating rabbits with washed hens' corpuscles, and the hens' corpuscles used for this purpose and for the hæmolytic tests were washed, after removal of the serum, three to four times with salt solution. As complement fresh guinea-pigs' serum was employed, the amount required being 0.05 cubic centimeter.

The quantities of each serum required to bind the complement in the presence of the vaccinial extract was determined, but in the table only the smallest quantity is given. The method of conducting the experiments was to mix the extract, calf's or ox's serum, and complement and add salt solution to bring the volumes uniformly to two cubic centimeters. The tubes were then placed in the thermostat for one hour, after which the hens' corpuscles and rabbits' immune serum, the latter diluted uniformly to 0.5 cubic centimeter, were added. After shaking to secure mixture of all ingredients, the tubes were returned to the thermostat for two hours, and then placed in the refrigerator for eighteen hours. The accompanying table gives in brief the results of the experiments. The results were, however, repeated many times with different extracts and sera of vaccinated calves.

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[11] Metschnikoff, *Annales de l'Institut Pasteur*, 1899, xiii, 737.

[12] Korschun and Morgenroth, *Berliner klin. Woch.*, 1902, xxxix, 870.

TABLE

| Immune serum No. 1 | Immune serum No. 2 | Normal calf serum | Normal ox serum | Guinea-pig complement | Vaccine extract | Anti-serum (hen) | 10% hens' corpuscles | |
|---|---|---|---|---|---|---|---|---|
| 0.05 | | | | 0.05 | 0.3 | 0.01 | 0.5 | 0 |
| | 0.033 | | | " | " | " | " | 0 |
| | | 1 c.c. | | " | " | " | " | + |
| | | | 0.7 c.c. | " | " | " | " | + |

*Controls.*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 0.05 | 0.5 | 0.01 | 0.5 | + |
| 0.5 | | | | " | 0 | " | " | + |
| | 0.5 | | | " | 0 | " | " | + |
| | | 1 c.c. | | " | 0 | " | " | + |
| | | | 0.7 | " | 0 | " | " | + |
| 0.5 | | | | 0 | 0 | 0 | " | 0 |
| | 0.5 | | | 0 | 0 | 0 | " | 0 |
| | | 1 c.c. | | 0 | 0 | 0 | " | 0 |
| | | | 0.7 | 0 | 0 | 0 | " | 0 |
| | | | | 0.05 | 0 | 0.01 | " | + |
| | | | | | 0.5 | 0 | " | 0 |
| | | | | | | 0.01 | " | 0 |

As the tabulated results show, the quantity of immune bodies present in the serum of vaccinated calves is small, but since no bodies acting in the same manner as these were ever detected in the serum of non-vaccinated calves, and no inhibition of hæmolysis was produced by ox serum when used in amounts below o.8 cubic centimeter, the conclusion is warranted that the serum of animals which have recovered from vaccinia contains specific antibodies capable of uniting with certain specific substances contained in extracts of vaccine pustules. In so far as our present knowledge permits us to account for the peculiar complement binding described above, we are justified in regarding the antibodies of the serum of vaccinated calves as of the nature of immune bodies. It is hoped that the results of the study of vaccinia may soon be extended to the study of variola.

The work described in this paper was begun in the Pathological Laboratory of the Johns Hopkins University. I wish, therefore, to express my indebtedness, for many courtesies, to Professor Welch and Dr. Ford.

# AN EXPERIMENTAL RESEARCH INTO THE RESUSCITATION OF DOGS KILLED BY ANESTHETICS AND ASPHYXIA.

By GEORGE CRILE, M.D., AND DAVID H. DOLLEY, M.D.

(*From the Laboratory of Surgical Physiology, Western Reserve Medical School, Cleveland.*)

PLATES XLII–XLIX.

In a previous communication by one of us (Crile) resuscitation was attempted by means of both direct and indirect cardiac massage, with and without artificial respiration, with and without intravenous saline infusion, and with and without the addition of adrenalin. The results of these experiments may be summarized as follows: By cardiac massage alone animals were rarely resuscitated at any time after quiescence of the circulation and respiration; by combining either direct or indirect cardiac massage with artificial respiration and the head-down posture a certain percentage of the animals were recovered after the lapse of from one to three minutes. The results, however, were quite uncertain, and in the case of death from chloroform recovery was the exception. By adding to cardiac massage artificial respiration and intravenous saline infusion, resuscitations were in a slightly greater proportion successful. The same procedures, with the addition of adrenalin to the intravenous saline infusion, were markedly more successful in the deaths from asphyxia through rarely so in the cases of death from chloroform. It required in almost every instance a vigorous compression of the thorax over the heart for a considerable time, varying from five to ten minutes, before resuscitation could be accomplished. Even then there were a considerable number of failures. In the majority of these failures autopsy showed the heart to be in a state of marked distension, indicating complete cardiac paralysis; not infrequently when results were delayed, the acute cardiac dilatation was partly relieved by venesection and by placing the dog in the inclined head-up posture, thereby partly relieving the paralyzed heart of its over-distension.

In this research the problem was approached from the arterial side, with the following physiologic factors as a basis: The physiological researches of Ringer, Kuliabio, Sollman, and others had shown that the excised heart could be made to beat again, even many hours after excision, when the coronary arteries were subjected to a considerable pressure from some circulating medium. The researches of Sollman showed that the *inauguration* of the beat was more dependent upon the physical factor of the increased pressure in the coronary arteries than upon the quality of fluid producing such pressure. He was able to inaugurate cardiac beats by perfusing the coronaries with metallic mercury. The basic problem, then, in resuscitation seemed to us to be that of securing by means of some infusion a coronary pressure approximately amounting to from thirty to forty millimeters of mercury. It was impossible to raise the coronary pressure in the intact animal to this height by means of cardiac massage alone; it was almost impossible to raise it by the mechanics of infusion alone, and generally impossible to raise it to this height by means of cardiac massage and plain infusion combined.

The value of adrenalin in raising the blood pressure, by its action upon the vascular walls in the state of suspended animation, has been heretofore thoroughly established. Introducing the adrenalin into the venous circulation, while easy and practical, had the following disadvantage: the adrenalin first came in contact with the vessels having the least power of influencing the blood pressure, and before a material rise could be effected by its action upon the arteries it was necessary that the solution should pass through the right heart, the lungs, then back to the left heart on its way to the aorta, thence affecting directly the coronary artery. In the previous research it was found that this too often caused an accumulation of solution and blood in the dilated, paralyzed chambers of the heart, defeating resuscitation.

It seemed reasonable to us to suppose that the most direct and effective way of producing a coronary pressure amounting to thirty or forty millimeters of mercury was by introducing a solution of adrenalin into the arterial system toward the heart. In this way the moment the adrenalin was introduced it caused a contraction of the strong arterial walls, beginning to produce an arterial pressure which would communicate directly with the coronary artery without first passing through the already distended and paralyzed chambers of the heart and through the lungs. These considerations were particularly impressed upon us by a clinical case which has elsewhere been reported.

Sixty experiments were made in which the tracings were preserved and the records noted. In the recovery experiments the greatest care was taken to secure for the animals all the comfort possible, and as few of this class of experiments were made as was consistent with the collection of data sufficient for our conclusions.

Three groups of cases were investigated: (1) death from asphyxia, (2) from ether, (3) from chloroform. Inasmuch as the methods and results were quite similar, they may all be described together.

We first tested the method as to its usefulness in raising the blood pressure, and found that by tying a cannula into an artery toward the heart and introducing through it a solution of adrenalin the blood pressure was immediately raised, the latent period being distinctly less than when the solution was introduced intravenously. In fact, we may say that there was no latent period, the blood pressure rising instantly. It was found, too, that the most effective method of raising the blood pressure was by the injection of the full strength of 1–1000 solution of adrenalin by means of a hypodermic syringe into the rubber tubing near the cannula. The results in these experiments showed a distinctly more powerful action than in the experiments in which the solution of adrenalin was previously made and poured into the funnel.

The first experiment was made upon an animal killed by chloroform. A cannula was inserted in the femoral artery directed toward the heart. After two minutes artificial respirations were begun and the saline solution was allowed to flow for perhaps ten seconds; then a hypodermic injection of from one to two cubic centimeters of 1–1000 solution of adrenalin was given into the tube near the cannula. A few seconds later the blood pressure began to rise steadily; then a few firm pressures upon the thorax over the heart caused a leaping up of high pulse waves, and at the end of three quarters of a minute the heart beat vigorously, driving the blood up into the infusion bottle, which was raised to a height of five feet. The saline injection and cardiac massage were discontinued, and in a few minutes irregular respirations slowly began, decreasing in force and increasing in frequency until the normal was established. The animal was then killed.

Following this experiment others were made, each time a longer period being allowed to elapse from the moment of death until the beginning of resuscitation. In determining the moment of death from chloroform it was found that by administering an excessive dose by inhalation the blood pressure sank with remarkable rapidity to the abscissa line, but that after this in forty per cent. of such deaths feeble pulse waves reappeared after periods varying from a few seconds to over three minutes, the blood pressure rising to from ten to thirty millimeters, then gradually declining, the pulse wave progressively diminishing in length. After a period of resumption of beat varying from a few seconds to six minutes, and averaging two minutes, the pressure usually fell to the abscissa line and no further waves were recorded upon the drum. It was only after the pressure had reached the abscissa line, after all pulse waves had ceased, and no heart sound, however feeble, could be auscultated, that we recorded the moment of death. The time was then extended to five, ten, fifteen, twenty, and thirty minutes in adult dogs, and in puppies up to an hour.

The limitations of the method soon appeared. Among them were the following: (a) ante-mortem clotting, (b) over-distension of the heart, (c) later failure of the heart, circulation, and respiration, (d) the apparently imperfect recovery of the brain.

*Ante-mortem Clotting.*—When a considerable time had elapsed, say ten minutes or more after death, and particularly if there had been some delay in resuscitation of the heart, thereby inducing vigorous and protracted massage, clotting occurred more frequently. Direct cardiac massage was then made, which seemed further to facilitate clotting. In other cases indirect massage was made with extreme vigor, showing like results. Finally, an animal was etherized to the point of respiratory failure, then sustained with a very low blood pressure by means of artificial respiration combined with vigorous cardiac massage, for a period of about seven minutes, after which the heart was opened before it had ceased beating and small clots were found. No infusion in this case had been given and no other operation was performed. This seemed to confirm our previous conclusion that either vigorous or prolonged massage of the heart predisposed to ante-mortem clotting and that direct massage of the heart favored clotting more than indirect massage by rhythmic pressure upon the intact thorax over the heart.

It was found that animals could be resuscitated after several compressions of the chest by waiting a little longer for the rise in the pressure from the adrenalin infusion alone. It was obvious that in our previous experiments we gave unnecessary massage as to force, frequency, and duration. This is the more reasonable since the object of the massage is not that it will materially aid the heart in *inaugurating* its rhythmic contractions, but that it will distribute the fluid through the circulation which has been injected by gravity alone. Pressing upon the thorax itself, would effect a certain amount of circulation, if no heart existed and a system of vessels, one having valves and the other not, replaced it, and in the dead animal pressure upon the thorax alone is capable of producing an artificial circulation. This is by no means accomplished by its action upon the heart solely, but by its action upon all the large vessels—arteries, veins, and capillaries together.

We also observed what on *a priori* grounds we supposed to be true, namely, that a tendency to clotting was increased in a direct ratio with the lapse of time. When clotting occurred resuscitation was practically always impossible. The heart would beat for a short time, but the beat would soon fade away.

*Over-distension of the Heart.*—In the unsuccessful cases, particularly those in which a considerable amount of infusion was given, the heart at autopsy was found over-distended. This over-distension was due to two factors; the first was the inevitable accumulation of blood in the heart during its gradual failure either in asphyxia or following chloroform and ether; second, the infused fluid passed through the coronary arteries, through the coronary vein into the right side of the heart. The left heart was not generally so distended as the right heart. It is obvious that if for any reason resuscitation is not promptly made the accumulation of fluid in the right heart must not only be considerable but must occur under pressure; since the valves prevent the escape of the fluid toward the periphery of the veins the accumulation must be largely in the heart itself, and when this condition is reached a peculiar vicious circle has been established. The greater the efforts at resuscitation by increasing the infusion the greater the distension of the paralyzed ventricle and the more certain is the failure of the attempt. In some instances after the chest was opened and the heart on inspection was found distended, rhythmic contractions of the heart began; on opening the pericardium in one instance a splendid resuscitation (Fig. 6.) was made.

*Later Failure of the Heart, Circulation, and Respiration.*—In a number of instances after a temporary resuscitation the circulation and the respiration failed, after which a second attempt at resuscitation was useless. In these cases the failure was generally gradual and occurred after the lapse of a varying period of time, ranging from three and a half to twenty hours. In these cases for the most part ten minutes or more had elapsed before resuscitation was attempted. A few animals in which resuscitation was attempted in less than ten minutes also showed later failure. We are not prepared to state the exact cause of this later failure.

*The Apparently Imperfect Recovery of the Brain Proper.*—In resuscitations after ten minutes, particularly those after from fifteen to twenty minutes, it was noted that while the circulation and the respiration were satisfactory the animal did not regain consciousness; the reason for this we are not at present prepared to state, but it will be further investigated.

We also investigated the influence of prolonged chloroform anesthesia prior to death. Animals were kept under chloroform anesthesia for an hour and then killed by an over-dose. They were resuscitated perhaps a little less readily than the animals killed immediately by an over-dose, though their resuscitation was, up to ten minutes, satisfactory.

*Hirudin.*—After encountering the difficulty with clotting in the delayed cases, we tested the value of hirudin in the prevention of clotting. In the cases in which hirudin was given prior to death no clotting occurred in a single instance in the heart or blood-vessels. In these cases, which were limited to puppies not over three months old, we are, however, inclined to believe that œdema of the lungs was distinctly more marked. The blood seemed to lose its viscosity and the œdematous fluid accumulated rapidly in the bronchial tubes, and finally flowed out of the trachea, presenting a reddish tinge. In the successful cases this always occurred before the inauguration of the heart-beat. There was in addition free blood-tinged fluid in the thorax. While hirudin, in the case of puppies at least, entirely obviates the clotting factor it introduces an equally fatal obstacle, *i. e.*, waterlogging of the thorax, which we were not able to prevent by reducing the infusion pressure to a minimum consistent with its efficacy.

*Puppies.*—The experiments on puppies showed at once that resuscitation was more readily done, and successful after a longer period after death, than in the adult dog. In three instance thirty-five minutes after death a fairly good circulation was obtained (Fig. 8).

*Solution Employed for Infusion.*—The observations in this series as well as in other researches within the past year have led us to conclude that Ringer's solution yields more favorable results than saline solution. The most satisfactory results were obtained with the infusion bottle at a height of five or six feet.

*Conclusions.*—Based on the data supplied by these researches and those of preceding researches on similar lines we arrive at the following conclusions:

(1) Animals after death from chloroform, ether, or asphyxia up to five minutes were uniformly and readily resuscitated,

provided that the full techinque which has been described was applied; up to ten minutes there was an occasional failure; beyond ten minutes consciousness was rarely restored, and the proportion of successes in the resuscitation of the circulation and the respiration diminished with the lapse of time; after twenty-three minutes in adult dogs and thirty-five minutes in puppies complete return of the circulation was not accomplished.

(2) After death from chloroform and ether animals were more readily resuscitated than after death from asphyxia.

(3) Resuscitation, if successful, occurred within one minute after the administration of adrenalin in the majority of instances; it rarely occurred after an interval greater than three minutes.

(4) The greatest difficulty to be overcome was clotting. The probability of clotting increased in direct ratio with the lapse of time after death. Cardiac trauma predisposed to clotting.

(5) The younger the animal, up to certain limits, the more readily it was resuscitated.

(6) Any artery may be used for the infusion, though the advantage is probably slightly in favor of the carotid. The infusion should be directed toward the heart.

(7) The probable success of resuscitation is greater in inverse relation to the lapse of time after death; a rapid rise in the arterial pressure is attained by arterial infusion with a therapeutic dose of adrenalin, together with good artificial respiration and the avoidance of unnecessary cardiac trauma by massage. All artificial aids should cease as soon as the functions are competent.

## ILLUSTRATIVE PROTOCOLS.

EXPERIMENT IV. *The effect of time and of massage; on the formation of clots.* Mongrel mastiff, 20 K. Chloroform administered until death.

10.09—Respiration stopped.

10.14—Pulse was not palpable at carotid.

10.15—Heart sounds ceased. The opened carotid bled for some time with a non-pulsating drop flow which stopped when the head was raised. A tracheal cannula was rapidly adjusted, and with a manometer cannula in the left carotid a small cannula in the right carotid was connected with the saline infusion bottle at a height of seven feet. A pin-hole cannula in the left femoral vein was connected to an infusion bottle containing 1–100000 solution of adrenalin chloride at a height of three feet.

10.47 (32 minutes)—Resuscitation attempted. Cardiac massage was vigorous. Two cubic centimeters of the solution of adrenalin chloride were injecte into the infusion tubing. No results came from the first dose of adrenalin within two minutes. With a second dose there was a perceptible manometer pulse and measures were temporarily stopped, the blood pressure being 40 mm., but the level quickly declined to the abscissa. Further efforts, namely massage and a mass dose of adrenalin with a continuous 1–100000 infusion into the femoral vein, failed to improve the heart-beat and to raise the blood pressure. After some time, when no pulse could be felt, the carotid infusion was again started and a second time beats were evoked, but other measures failed to keep up the heart's activity. In the latter part of the experiment the dog's feet were lowered.

At autopsy the heart was full of bloody fluid, the pericardium was tense and the heart dilated. Clots were found in the heart.

EXPERIMENT XXVIII. *Resuscitation six minutes after death.* (Fig. 1.) Ether anesthesia. This experiment was performed with moderate aseptic precautions.

10.59—Control blood pressure 156 mm.

11.00—Began to force ether.

11.04—Respiration stopped, after which cardiac failure gradually ensued.

11.06—Heart sounds ceased and pulse not palpable.

11.12—Resuscitation was begun (6 minutes). Saline infusion plus 2 c.c. of adrenalin chloride (1–1000) was administered through the axillary artery together with artificial respiration and gentle heart massage. About twenty respirations were given. The heart began to beat in about one and a half minutes. The blood pressure rose sharply to 240 mm., which level was sustained for several minutes. It gradually fell to 80 mm., at 11.24.

11.18—Spontaneous respiration began.

11.20—Ether was required and given. The respiration was at first very irregular and spasmodic, gradually becoming quiet and regular.

11.29—Blood pressure 130 mm.

11.40—Blood pressure 160 mm.

11.50—Blood pressure 160 mm. The trachea was sewed up, the wounds in the neck and axilla were closed with large gauze drainage and were bandaged. The dog was removed from the table and allowed to recover. Late in the afternoon the dog had considerable respiratory difficulty, apparently due to obstruction in the trachea. The animal was etherized, a blood clot was removed from the trachea, a cannula inserted, and the neck bandaged.

The next morning his condition was good and he was walking about, but as considerable pus was coming from the tracheal cannula it was decided to kill him. He was etherized and the carotid artery was again connected with the manometer.

10.30—Blood pressure 106 mm.

11.00—Blood pressure 112 mm.: the difference being due to the depth of anesthesia. The dog was then killed.

Beyond the infection about the trachea, there were no macroscopic lesions found at autopsy.

EXPERIMENT XIX. *Resuscitation five minutes after apparent death.* (Fig. 2.) Mongrel in rather poor condition; weight 7.3 K. Anesthetized with ether.

10.35—Control blood pressure 140 mm.

10.42—Trachea clamped. Several control determinations of blood pressure showing stages of asphyxia were made.

10.15—Anesthetized with chloroform.

11.13—Chloroform forced (anesthesia during one hour).

11.17¾—Heart stopped before respiration according to manometer tracing.

11.19—Heart-beats recurred for about ten seconds.

11.20—Heart-beats recurred a second time, about twelve in all being recorded.

11.20½—Heart sounds ceased and the pulse was not palpable.

11.40½ (20 minutes)—Resuscitation started. Infusion of Ringer's fluid through axillary artery, plus 2 c.c. of solution of adrenalin chloride (1—1000); and artificial respiration were started simultaneously. Gentle pressure at intervals on the chest with the feet lowered was also used. After several minutes the axillary cannula pulled out and was refixed in the right carotid.

11.43 (23 minutes)—Resuscitation was again attempted, artificial respiration having been kept up continuously. In about a minute the heart was noticed to be beating. The blood pressure curve rose rather slowly to a height of 112 mm. with a very irregular pulse wave.

11.48—Respiration recurred.

11.49—Control: blood pressure level has fallen to 50 mm.

12.07—Control blood pressure 36 mm. Burning of paw evokes no reaction.

12.20—Blood pressure 50 mm. Pulse wave much improved. Faint corneal reflex in right eye.

2.00—Control blood pressure 54 mm. Respirations have become more normal in type, but the heart rate is distinctly lowered. Burning of paw evokes a very slight rising of blood pressure only. Knee jerks are present in both hind legs. Corneal reflex is present in the right eye, though feebler than normal; it is just perceptible and delayed in the left. Dog appears insensible to touch sensation, but burning anus causes spontaneous movements of the legs. The pupils do not react to light. The right pupil is more dilated than the left.

4.00—Control, showing rise of pressure due to accidental asphyxia.

4.30—Control blood pressure fallen to 28 mm.

Ante-mortem examination was performed. No clot appeared in the heart and the viscera were apparently normal.

EXPERIMENT XLVIII. *Partial resuscitation of a puppy thirty minutes after apparent death.* (Fig. 7.) Skye terrier puppy, three months old, weight 2 K.; anesthetized with ether. A full dose of hirudin—10 c.c. of a .4 of 1% solution —was given to obviate clotting.

2.55—Control blood pressure 104 mm. Chloroform exchanged for ether and forced.

3.03½—Respiration stopped. Control.

3.06—Heart sounds, then pulse, not perceptible.

3.36 (30 minutes)—Began resuscitation. Infusion of Ringer's solution from the height of four feet, plus 2.5 c.c. of 1—1000 adrenalin through carotid artery, with artificial respiration and gentle heart massage, were started simultaneously. Heart began to beat in about two minutes. Infusion was stopped as soon as the blood pressure began to rise decidedly. In about three minutes the blood pressure reached a height of 94 mm., with a regular and rhythmic heart-beat of good strength.

3.41—Drum stopped.

3.50—Control blood pressure 102 mm.

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PLATE XLIII.

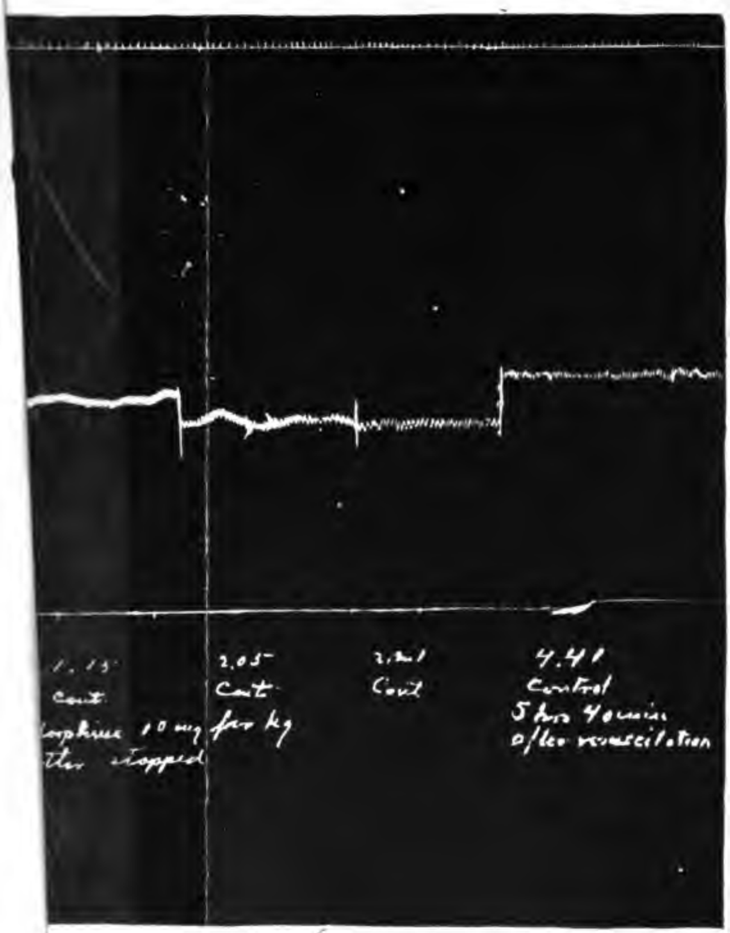

1.15
Cont.
orphine 10 mg per kg
ther stopped
2.05
Cont.
2.51
Cont
4.41
Control
5 hrs 40 min
after resuscitation

PLATE XLV.

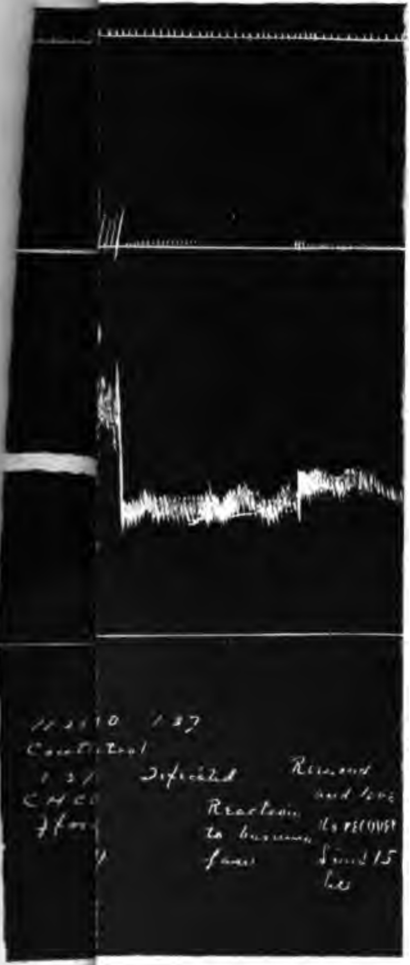
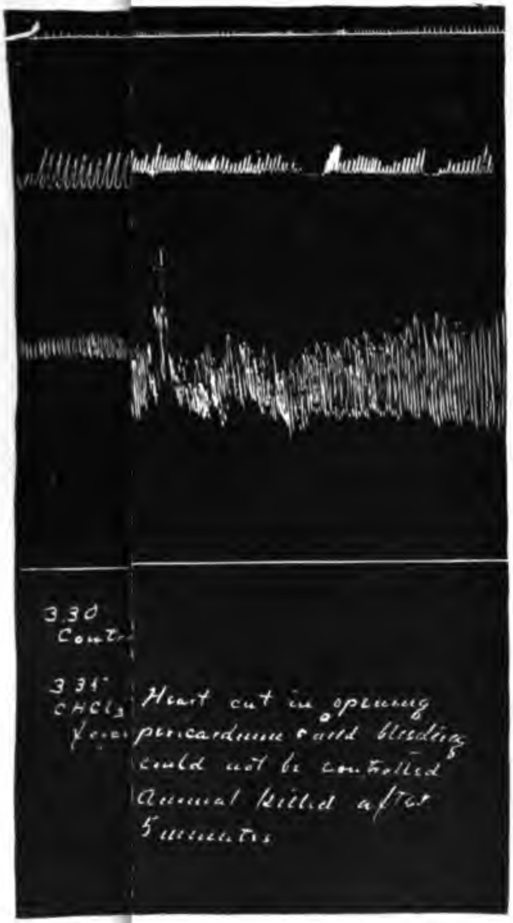

10 1 37
Defecated
Reaction
to burning
flame
Removed
and
to recover
15

PLATE XLVII.


3.30
Cont
3 35
CHCl3
Heart cut in opening of pericardium and bleeding could not be controlled
Animal killed after 5 minutes

## PLATE XLVIII

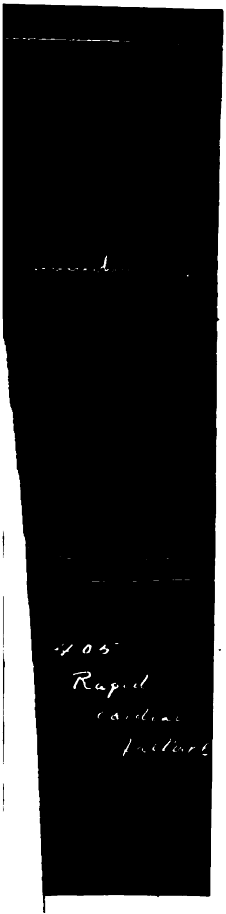


PLATE XLIX.

3.59—There are three feeble spontaneous respirations which the control shows. These occurred after several attempts to induce them by stopping artificial respirations, burning anus, and pulling on the tongue. Artificial respiration was continued, but the heart began at once to fail.

4.05—Heart stopped beating entirely.

Complete resuscitation within six minutes has been obtained in a number of experiments since the foregoing was sent to press. Later experiments have apparently established the unequal susceptibility for resuscitation of various parts of the brain.

# THE THERMOSTABILE ANTICOMPLEMENTARY CONSTITUENTS OF THE BLOOD.

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The present article deals with the antihæmolytic principles the blood, and especially of normal blood serum, which act against that form of hæmolysis caused by the amboceptor-complement complex.

The present state of our knowledge permits us to divide the antihæmolytic principles of normal blood into two classes. The principles of the first class are directed against certain definite hæmolysins—glucosides, mercuric chloride, agaricin—and certain hæmolytic toxins of micro-organic origin (bacterial toxins). The principles of the second class are directed against the more complex hæmolysins as they exist in normal and immune sera. Preliminary to the consideration of the immediate subject of this paper I will enumerate the factors, other than those which come into play in connection with the normal serum which influence the process of hæmolysis.

The hæmolytic process can be altered and reduced in different ways. Temperatures above 50° C. render all complements with few exceptions [1,2] inactive. Divers micro-organisms,[3] organic tissues,[4] certain tissue constituents,[5] and the complex antigen-antibody to which belong the complement fixation of specific precipitates [6-12] all reduce or prevent hæmolysis by interfering under certain conditions with the union of complement and hæmolytic amboceptor. Bordet[13,14]

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[1] Ehrlich and Morgenroth, Ueber Hämolysine, Zweite Mittheil., *Berl. Klin Woch.*, 1899, xxxvi, 481.

[2] Noguchi, On the heat lability of the complements of cold-blooded animals, *Centralbl. f. Bakteriologie*, 1903, xxxiv, 283.

[3] M. Wilde, Ueber die Absorption der Alexine durch abgetötete Bacterien, *Berl. klin. Woch.*, 1901, xxxviii, 878.

[4] V. Dungern, Beiträge zur Immunitätslehre, *Münch. med. Woch.*, 1900, xlvii, 677.

ad Ehrlich and Morgenroth [13] describe anticomplements in the strict sense, he existence of which is denied by Moreschi, who accounts for the effect ascribed o them by the complement binding power of specific precipitates.

Ehrlich first described in relation to tetanolysin the antihæmolytic property f normal serum, and Kraus and Clairmont,[16] found this property to be exerted gainst many bacterial hæmolysins. Neisser and Wechsberg [17] observed he neutralization of staphylolysins by normal guinea-pig serum. A great advance was made by Ransom's [18] discovery of the antihæmolytic action of cholesterin upon saponin. Noguchi,[19] following upon Ransom's discovery, found that cholesterin prevented hæmolysis by agaricin and tetanolysin. These observations drew active attention to the part played by the lipoids of the blood in relation to hæmolysis. P. Th. Müller,[20] Kyes and Sachs,[21] Landsteiner and v. Eisler,[22] Pascucci,[23] studied the nature of the antihæmolytic bodies of normal serum. Müller confirmed the antihæmolytic property of cholesterin. Kyes and Sachs found that cholesterin has no antihæmolytic power against staphylolysin and v. Eisler [24] located the antistaphylolytic principle in the globulin fraction. Pribram [25] found that cholesterin is active against the Nasik vibriolysin; and E. Abderhalden and E. R. Le Count [26] attempted to locate the groups upon which depend the antihæmolytic properties of cholesterin, finding that the free hydroxyl group has a certain relation to these properties. Detre and Sellei [27] stated that lecithin is capable of preventing sublimate hæmolysis.

The investigations summarized indicate that the normal serum contains definite chemical substances which can prevent the hæmolysis caused by bacterial toxins, mineral chemicals, and certain vegetable principles of definite composition. Some differences of opinion exist regarding the nature of the inhibiting substances. Undoubtedly the lipoids of the serum as represented by cholesterin and lecithin possess this property. Apparently a similar property resides in the proteids of the serum. In regard to the latter the question which would at

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[5] P. A. Levene and E. R. Baldwin, On the antihæmolytic action of some cell and tissue constituents, *Jour. of Med. Research*, 1904, xii, 205.

[6] Gengou, Sur les sensibilisatrice des serums actifs contre les substances albuminoides, *Ann. Inst. Pasteur*, 1902, xvi, 734.

[7] F. P. Gay, The fixation of alexines by specific serum percipitates, *Centralbl. f. Bakteriologie*, 1905, xxxix, 603.

[8] Moreschi, Zur Lehre von den Antikomplementen, *Berl. klin. Woch.*, 1905, xlii, 1181.

[9] Neisser and Sachs, Ein Verfahren zum forensischen Nachweise der Herkunft des Blutes, *Berl. klin. Woch.*, 1905, xlii, 1388.

[10] Pfeiffer and Moreschi, Ueber scheinbare Antikomplementär- und Antiamboceptorwirkung precipitirender Sera im Thierkörper, *Berl. klin. Woch.*, 1906, xliii, 33.

[11] Liefmann, Ueber die Komplementablenkung bei Precipitationsvorgängen, *Berl. klin. Woch.*, 1906, xliii, 448.

[12] Muir and Martin, On the deviation of complement by a serum and its antiserum and its relations to the precipitin test, *Jour. of Hygiene*, 1906, vi, 265.

[13] Bordet, Les serums hémolytiques et leurs antitoxines, *Ann. Inst. Pasteur*, 1900, xiv, 257.

once arise is whether all the lipoids had been separated from the proteid fraction of the serum.

A second class of antilytic substances, present in normal serum, are the d antagonists of complements and amboceptors. Camus and Gley[28] found that heating eel and other normal sera to 56° C. develops in them an antihæmolytic property which they regard as acting antagonistically to the lytic principles. Müller observed this behavior of sera heated to 56° C. and, in addition, the antilytic property exerted by certain unheated sera. Ehrlich and Sachs[30] and Sachs[31] found that at 60° C. but not at 50° C. dog serum becomes anticomplementary. According to their view the zymotoxic group, but not the haptophoric, is destroyed by the higher temperature whereby complementoid is produced. Heating does not, however, leave the haptophore group intact, since complementoid exerts weaker affinity for amboceptor than the original complement. Although dog serum is not rendered anticomplementary when heated to 49° to 50° C. yet it is rendered inactive. No adequate explanation of this inactivity has been advanced. Ox serum was also observed to become anticomplementary when heated to 60° C., but the existence of complementoid could not be demonstrated because no union between its amboceptor and guinea-pig corpuscles occurs in the absence of normal complement (horse). Recently Bordet and Gay[33] have shown that other factors operate in this phenomenon. Guinea-pig corpuscles can really be sensitized by ox amboceptor, but when the excess of inactive ox serum is removed, horse complement does not exert a dissolving action on the corpuscles (although guinea-pig complement dissolves them promptly). Hence they assert that inactivated ox serum contains bodies which energize the power of horse serum. Neisser and Döring[34] observed in a case of uraemia that the serum on heating to 56° C., became anticomplementary. Neisser and Friedemann[35] made a similar observation, and in addition they found that heating to 57° C.

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[14] *Idem*. Bemerkung über die Antikomplemente, *Berl. klin. Woch.*, 1906, xliii, 17.

[15] Ehrlich and Morgenroth, Ueber Hämolysine, Vierte Mitth., *Berl. klin. Woch.*, 1900, xxxvii, 681.

[16] Kraus and Clairmont, Ueber Bacteriohämolysine und Antihämolysine *Wien. klin. Woch.*, 1901, xiv, 1016.

[17] Neisser and Wechsberg, Ueber das Staphylotoxin, *Zeitschr. f. Hygiene*, 1901, xxxvi, 299.

[18] Ransom, Saponin und sein Gegengift, *Deut. med. Woch.*, 1901, xxvii, 194.

[19] Noguchi, The antihæmolytic action of blood sera, milk, and cholesterin upon agaricin, saponin, and tetanolysin, *Centralbl. f. Bakteriologie*, 1900, xxxii, 377.

[20] P. Th. Müller, Geht das Tetanolysin mit den Proteiden des Serums und des Eiklares eine ungiftige Verbindung ein? *Centralbl. f. Bakteriologie*, 1903, xxxiv, 567.

[21] Kyes and Sachs, Zur Kenntniss der Cobragift activirenden Substanzen *Berl. klin. Woch.*, 1903, xl, 59, Footnote 3.

[22] Landsteiner and v. Eisler, Ueber Agglutinin-und Lysinwirkung, *Centralbl. f. Bakteriologie*, 1905, xxxix, 309.

[23] Pascucci, Die Zusammensetzung der Blutscheibenstromes und die Hæmilyse, *Hofmeister's Beiträge*, 1905, vi, 552.

tivated the serum without destroying the anticomplementary action. They avored to account for the phenomenon by supposing the amboceptor of mic blood to be less heat stabile than normal, whereby, at 56° C., the cytophy-group is destroyed and the complementary one is left intact (amboceptoid) capable of deviating complement. They thought to prove their view by nting out that extraction of the heated serum by susceptible red corpuscles not remove the inhibitory action of the serum. Unfortunately, they seem t to have tested the corpuscles for resistance. Laqueur[36] confirmed the rk of these investigators and found, in addition, not only that similar condi-ns existed in the blood in other pathological states, but that they may be sent from uraemic blood. Senator[37] failed to find the phenomenon in classical amples of uraemia, and Bergmann and Keuthe[38] ascertained that it is irregular its occurrence in uraemia and occurs in other pathological conditions. That bbit serum may, after heating to 56° C., develop anticomplementary action is hown by certain experiments of Sachs[39] and Pfeiffer and Friedberger.[40] Muir nd Browning[41] mention that guinea-pig serum, from which complement has een removed by means of sensitized corpuscular stromata, previously heated o 56° C. over night and washed, does not become anticomplementary upon eating to 55° C., because no complement exists to be changed into complementoid. Manwaring[42] describes a qualitative change in heated immune serum after digestion with corpuscles, but does not define accurately this alteration.

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[24] v. Eisler, Bedeutung der Lipoide für die antihämolytische Wirkung des Blutes, *Zeitschr. f. exper. Pathologie und Therapie*, 1906, iii, 296.

[25] Pribram, Ueber Bakteriohämotoxine (Lysine) und Antihämotoxine, Handbuch der path. Mikroorganismen, 1906, Ergänzungsband, i, 291.

[26] E. Abderhalden and E. R. Le Count, Die Beziehungen zwischen Cholesterin, Lecithin, and Cobragift, Tetanustoxin, Saponin und Solanin, *Zeitschr. f. exper. Pathologie und Therapie*, 1905, ii.

[27] Detre and Sellei, Hämolytische Wirkung des Sublimates, *Wien. klin. Woch.*, 1904, xvii, 1195, 1234.

[28] Camus and Gley, A propos de l'existence, dans un serum sanguin, d'une action antagoniste de l'action hémolytique, *Compt. rend. d l. Soc. d. Biologie*, 1901, liii, 732.

[29] P. Th. Müller, Ueber die Antihämolysine normaler Sera, *Centralbl f. Bakteriologie*, 1901, xxix, 860.

[30] Ehrlich and Sachs, Ueber den Mechanismus der Amboceptorenwirkung *Berl. klin. Woch.*, 1902, xxxix, 492.

[31] H. Sachs, Giebt es einheitliche Alexinwirkung? *Berl. klin. Woch.*, 1902, xxxix, 216.

[32] *Idem*, Ueber Komplementoide, *Centralbl. f. Bakteriologie*, 1905, xl, 125.

[33] Bordet and Gay, Sur les relations des sensibilisatrice avec l'alexine, *Ann Inst. Pasteur*, 1906, xx, 467.

[34] Neisser and Döring, Zur Kenntnis der hämolytischen Eigenschafte des menschlichen Serums, *Berl. klin. Woch.*, 1901, xxxviii, 593.

[35] Neisser and Friedemann, Ueber Amboceptoidbildung in einem urämischen Serum, *Berl. klin. Woch.*, 1902, xxxix, 677.

The difference in the heat-effects, according as the tempera to which the sera are exposed are about 50° C. or between and 60° C., is remarkable. If we assume, as Ehrlich and Sa have done, that complementoid is formed at 60°C., what are to infer happens at 50° C.? If the anticomplementary effec caused by destruction of the zymotoxic groups, what chang are produced at 50° C. leading to inactivation without develop any antagonistic activity? The view that the difference depen on the thermolability of the amboceptor is hypothetical, and experiments adduced by Neisser and Friedemann to uphold are unconvincing. I shall return to this point later in connecti with my experiments which seem to me to afford a solution the problem.

That the blood lipoids may antagonize hæmolysis by amboceptor-comple ments has also been shown. Landsteiner and v. Eisler[43] extracted lipoids fro blood corpuscles and noted that they exerted an antihæmolytic action agains serum hæmolysins. Later, these authors[44] found that blood serum contain similar bodies in smaller amounts than the corpuscles. They believed th action of the lipoids to be anti-amboceptoric, in the sense that they constit the receptors, which in the intact corpuscles anchor the amboceptors. Bodi having the same action can be obtained from the stroma of corpuscles. Bang and Forssmann[45,46] extracted ox corpuscles with ether and obtained a mixtu which, according to them, contains the hæmolysin or amboceptor-producing agent and antihæmolysin (complement-neutralizer). By treating the etherea extract with aceton they obtained in solution the latter principle; the insolub residue retained this amboceptor-producing body. This can be separated from phoshatides and cerebrosides by extraction with alcohol, ether, chlorofor or cold benzol. Bang and Forssmann regard the amboceptor-fixing bod (receptor in Ehrlich's sense) and amboceptor-producing substance as distinc from each other. Landsteiner and v. Eisler and Bang and Forssmann ob-

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[36] Laquer, Zur Kenntnis urämischer Zustände, *Deut. med. Woch.*, 1901, xxvii, 744.

[37] H. Senator, Ueber die hämolytische Eigenschaft des Blutserums bei Urämie, *Berl. klin. Woch.*, 1904, xli, 181.

[38] Bergmann and Keuthe, Die Hemmung der Hämolyse durch inactivirt menschliche Sera, *Zeitschr. f. exper. Pathologie und Therapie*, 1906, iii, 255.

[39] H. Sachs, Ueber das Zusammenwirken normaler und immunisatorisch erzeugter Amboceptoren bei der Hämolyse, *Deut. med. Woch.*, 1905, xxxi, 705.

[40] Pfeiffer and Friedberger, Beitrag zur Lehre von den antagonistischen Serumfunktionen, *Centralbl. f. Bakteriologie*, 1906, xli, 223.

[41] Muir and Browning, On the properties of anti-immune bodies and complementoids, *Jour. of Hygiene*, 1906, vi, 1.

[42] Manwaring, The absorption of hemolytic amboceptor, *Jour. of Infect. Diseases*, 1905, ii, 485.

ved that stromata of red corpuscles, freed of fat by solvents, are no longer le to fix amboceptor.

I wish, in this place, to point out that the anticomplementary ody of Bang and Forssmann is, according to their view, of pecific nature. It is questionable, indeed, whether their experi- nents wholly support this view (their experiments *l. c.* p. 261 an be interpreted so as to show the effect to be upon the ambocep- or rather than upon the complement). This body is ther- nostable: boiling for four minutes does not destroy it, but longer boiling does. It is of some interest to learn that these authors look upon this body as fixing the complement to the corpuscles after it has been exposed for action by the union of corpuscles and amboceptors. Hæmolysis results, therefore, from union of amboceptor and complement separately with the corpuscles.

A review of the literature upon the inhibition of serum hæmolysis brings out from a large mass of hypothesis a few definite facts. It is established that many normal and pathological bloods contain certain constituents which possess the power to suppress the action of serum-complements. This anticomplementary action may be present in the native serum or it may manifest itself only after the serum has been heated to 56° C. or higher. It has been shown that by extraction with certain solvents red corpuscles and serum yield substances which exert antilytic action. Whether the antilytic action of fresh and heated sera depend wholly, or at all, upon these substances is not proven beyond doubt. It is not proven that any of these antilytic substances are merely modified complements and amboceptors, and the existence of complementoid and amboceptoid has not been established. Apparently there are differences of considerable magnitude between the influences of heat and chemicals upon the antilytic action of sera and these extractives. It may be an

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[43] Landsteiner and v. Eisler, Ueber die Wirkungsweise hämolytischer Sera, *Wien. klin. Woch.*, 1904, xxvii, 676.

[44] *Idem*, Ueber Agglutinin- und Lysinwirkung, *Centralbl. f. Bakteriologie*, 1905, xxxix, 309.

[45] Bang and Forssmann, Untersuchung über die Hämolysinbildung, Vorl Mitth., *Centralbl. f. Bakteriologie*, 1905, xl, 150.

[46] *Idem*, Untersuchung über die Hämolysinbildung, *Hofmeister's Beiträge*. 1906, iii, 238.

accidental circumstance that the temperatures which de complement and amboceptor also remove this antilytic prop from the serum. The influence of temperature upon combina and dissociation of serum constituents is probably very w The different effects produced at 50° C. are cases in point. Ma waring[47],[48] found that normal goat serum, heated to 56° to 59°, acquired certain properties which he ascribed to a t serum component. The heated serum might be purely auxily or purely antilytic, or again, auxilytic in certain amounts a antilytic in others, or, even as a fourth possibility, complet inactive. Sera of different normal animals heated under th conditions might differ widely in the action of the third com ponents. No explanation was offered for these variable effec Possibly this effect is brought about by liberation of certai hæmolytic lipoids from inactive combinations, and these ma be powerful enough to overcome the antilytic action of th antilysins whose action we are considering. I have observe many instances of the auxilytic effect described by Manwaring but sera from which the lipoids have been extracted by alcoho and ether do not develop, on heating, auxilytic properties.

### NORMAL SERUM AS ANTIHÆMOLYSIN.

To bring out the antihæmolytic action of normal serum i is customary to inactivate it at 56° C. Either the heated serum and active hæmolytic serum are first mixed and the blood corpuscles added later, or, the heated serum and complement are first brought together and at a later stage the inactive hæmolytic serum (amboceptor) is added. Fresh serum can be tested accurately for antilytic action only when it does not contain suitable complement.

Table I shows the normal sera of the dog and sheep, heated to 56° C., to become antihæmolytic, while the same sera in the fresh state are non-protective. On the other hand, fresh ox serum protects its corpuscles, but heating to 56° C., removes the protective property. Inactivated ox serum is, however,

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[47] Manwaring, On auxilysins, *Jour. of Infect. Diseases*, 1906, iii, 225.

[48] *Idem*, The third serum component, *Jour. of Infect. Diseases*, 1906, iii, 645

# TABLE I.

THE ANTIHÆMOLYTIC POWER OF DOG, SHEEP, AND OX SERA WAS TESTED AGAINST THE ACTIVE ANTI-DOG (RABBIT), ANTI-SHEEP (GOAT), AND ANTI-OX (RABBIT) SERA. THE HÆMOLYTIC VALUES OF THESE IMMUNE SERA WERE, IN THE FRESH STATE, 0.05 C.C. FOR ANTI-DOG, 0.07 C.C. FOR ANTI-SHEEP AND 0.05 C.C. FOR ANTI-OX SERUM, UPON THEIR CORRESPONDING CORPUSCLES.

| Doses of the normal serum mixed with 0.2 of active immune serum. | Anti-dog serum 0.2 | | | | | | Anti-sheep serum 0.2 | | | | | | Anti-ox Serum 0.2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dog serum | | Sheep serum | | Ox serum | | Dog serum | | Sheep serum | | Ox serum | | Dog serum | | Sheep serum | | Ox serum | |
| | Fresh | 56° C | Fresh | 56° C | Fresh | 56° C | Fresh | 56° C | Fresh | 56° C | Fresh | 56° C | Fresh | 56° C | Fresh | 56° C | Fresh | 56° C |
| 1. | CH | none | CH | none | CH | none | CH | none | CH | none | almost CH | none | CH | none | slight | none | much | CH |
| 0.7 | " | " | " | trace | " | slight | " | none | " | " | " | slight | " | none | " | " | " | " |
| 0.5 | " | trace | " | much | " | almost CH | " | slight | " | " | " | " | " | slight | " | trace | CH | " |
| 0.3 | " | " | " | " | " | " | " | moderate | " | trace | " | much | " | " | almost CH | slight | " | " |
| 0.2 | " | " | " | almost CH | " | " | " | much | " | " | " | " | " | mod'te | CH | mod'te | " | " |
| 0.1 | " | " | " | " | " | CH | " | " | " | slight | " | almost CH | " | much | " | much | " | " |
| 0.07 | " | much | " | " | " | " | " | almost | " | mod'te | " | " | " | almost CH | " | almost CH | " | " |
| 0.05 | " | " | " | CH | " | " | " | CH | " | much | " | CH | " | CH | " | CH | " | CH |
| 0.03 | " | CH | " | " | " | " | " | " | " | CH | " | " | " | " | " | " | " | |
| O (control) | CH | CH | CH | CH | CH | CH | CH | CH | CH | CH | CH | CH | CH | CH | CH | CH | CH | |

protective for alien corpuscles. The heated sera are in gener antihæmolytic for many kinds of corpuscles, against several, a least, different kinds of actively hæmolytic sera. Table II bring out this fact, and it also shows that heated dog serum neutralize the action of fresh dog serum. There is, therefore, no indicatio of a specific antihæmolytic action of the heated sera.

TABLE II.

| Amount of heated normal serum added to 0.5 c.c. of fresh active dog serum | Sheep corpuscles | Ox corpuscles |
|---|---|---|
| Sheep serum (56° C) 1 c.c. | no hæmolysis | slight hæmolysis |
| Ox serum (56° C) 1 c.c. | moderate hæmolysis | moderate hæmolysis (in other instances none or complete). |
| Dog serum (56° C) 1 c.c. | moderate " | moderate hæmolysis. |
| 0.9% saline solution 1 c.c. | complete " | complete " |

## NATURE OF ANTIHÆMOLYTIC ACTION.

It is necessary to decide whether the antagonistic action is directed against complement or amboceptor. A hæmolytic combination of inactivated anti-sheep serum (from goat) and guinea-pig complement was prepared. An amount of 0.1 cubic centimeter of the former and 0.01 cubic centimeter of the latter caused prompt lysis of the sheep corpuscles. Now, to one cubic centimeter of each of the inactivated normal sera 0.01 cubic centimeter guinea-pig complement was added, the mixture being kept at 37° C. for one hour. The sheep corpuscles were then added, and after another half hour, 0.1 cubic centimeter of inactive anti-sheep serum. Reading was made at the expiration of twenty hours; the result was as follows:

Inactivated normal sheep serum (56° C.) 1 c.c. = No hæmolysis.
" " ox " " 1 c.c. = " "
" " dog " " 1 c.c. = " "
Control (no normal serum) saline 1 c.c. = Complete hæmolysis.

The corpuscles were now separated from the different mixtures, resuspended in two cubic centimeters of 0.9 per cent. saline solution, and 0.1 cubic centimeter guinea-pig complement added to each. The effect was as follows:

| | |
|---|---|
| Corpuscles treated with inactive sheep serum | = Marked hæmolysis |
| " " " " ox " | = " " |
| " " " " dog " | = Complete hæmolysis |

To each of the separated fluids 0.4 cubic centimeter amboceptor for sheep corpuscles was added; no hæmolysis was produced by these mixtures showing no active complement to be present. Hence it is concluded that the antihæmolytic action is due to neutralization of complement.

It may be stated, in this place, that the corpuscles separated from the inactive sera do not undergo lysis as readily as the controls without serum. The latter underwent lysis with 0.01 to 0.02 cubic centimeter guinea-pig serum, while the former required 0.1 cubic centimeter, which amount produced incomplete lysis in some cases. This result might be taken to indicate that the action is anti-amboceptoric, a conclusion which later experiments disproved. Mere digestion of corpuscles in inactive sera raises the resistance to lysis of the corpuscles. The anti-complementary action of the heated sera is also brought out by the next experiments.

| | |
|---|---|
| Anti-sheep amboceptor (goat) heated to 50° C. | 0.4. c.c. |
| Dog serum heated to 70° | 0.5 c.c. |

Kept one hour at 37° C., after which were added sheep corpuscles and 0.02 c.c. guinea-pig serum. Result: Complete hæmolysis.

| | |
|---|---|
| Guinea-pig serum | 0.2 c.c. |
| Dog serum heated to 70° C. | 0.5 c.c. |

Kept one hour at 37° C., after which sheep corpuscles and finally anti-sheep amboceptor 0.4 c.c. were added. Result: No hæmolysis.

### INACTIVATION AND ANTILYSIN OF NORMAL SERA.

That temperatures approaching 60° C. rendered the majority of normal sera antihæmolytic, while somewhat lower temperatures (49° to 50° C.) merely inactivated them, has been established by Müller, Ehrlich and Sachs, Neisser and Friedemann, and others.[49] The changes in the serum through which an antilytic property is developed is considered by Ehrlich and Sachs to be due to the formation of complementoid and by Neisser a Friedemann of amboceptoid. My own experiments have sho heated sera to be anticomplementary. The next experime refer to the temperatures at which the antilysin develops.

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[49] *Op. cit.*

Normal sheep, dog, and ox sera were tested against anti-shee anti-dog, and anti-ox sera. The anti-sera were prepared i rabbits. The anti-sera were inactivated at 50° C. and reactivat with guinea-pig serum. The titres of the anti-sera were abs equal; 0.1 cubic centimeter anti-sera and 0.02 cubic centime guinea-pig complement was needed for complete hæmolys The normal sera and complement were first mixed and aft the usual interval amboceptor and corpuscles were added Table III shows that none of the normal sera except the of the ox acting on its own cells possesses in the fresh state any antilytic action; these sera heated to 50° C. develop sometimes antilytic properties, while a temperature of 60° C. brings out, almost uniformly, this activity. The absence in normal sera of antilytic power may be due to a masking of antagonistic action by interaction of suitable complements and amboceptors but judging from the effect of the temperature of 50° C. it is more probable that the antilytic principle is present in the sera but is prevented from reacting with complements. If we turn now to a consideration of the action of normal sera in these several states upon a normal serum hæmolysin we shall see a considerable difference of effects. The antilytic action of sheep, ox, and dog sera was studied upon normal dog serum, the complete hæmolytic power of which was for ox corpuscles 0.35 cubic centimeter and for sheep corpuscles 0.25 cubic centimenter. Table IV exhibits the results and is to be compared with Table III.

The different effects brought out in Tables III and IV are considerable. Fresh normal serum of sheep and ox protect their own and each other's corpuscles from lysis by dog serum, but they are able only after heating to 56° C. or higher to protect these corpuscles from lysis by immune sera. In what does this distinction exist? Are the lysins of normal and immune sera distinct and do the normal antilysins act upon one set of lysins only, or are they identical, and do differences in power introduce ιew factor? The fact that heating the normal sera abolishes e differences in effect seems to point to a similarity in nature the antilysins acting upon normal and immune sera. ut in this case the antilysins must exist in different states in

TABLE III.

| umune amboceptor + Complement. | Sheep serum 0.8 c.c. | | | | Dog serum 0.8 c.c. | | | | Ox serum 0.8 c.c | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fresh | 50° C | 60° C | No serum | Fresh | 50° C | 60° C | No serum | Fresh | 50° C | 60° C | No serum |
| sheep amboceptor 0.1 a pig complement 0.02 | CH | moderate | none | CH | CH | CH | trace | CH | CH | CH | moderate | CH |
| dog amboceptor 0.2 ea pig complement 0.02 | CH | CH | trace | CH | CH | much | none | CH | CH | CH | moderate | CH |
| ox amboceptor 0.1 ea pig complement 0.05 | CH | much | slight | CH | CH | CH | none | CH | slight | moderate | CH* | CH |

lightly quicker than in absence of serum.

TABLE IV

| Dog serum and corpuscles. | Sheep serum 1.0 c.c. | | | | Ox serum 1.0 c.c. | | | | Dog serum 1.0 c.c. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fresh | 50°C | 60C° | No serum | Fresh | 50°C | 60°C | No serum | Fresh | 50°C | 60°C | No serum |
| Dog serum 0.25 vers. Sheep corpuscles | none | none | none | CH | trace | trace | trace | CH | CH | CH | none | CH |
| Dog serum 0.35 vers. Ox corpuscles | none | none | none | CH | none | trace | CH | CH | CH | CH | trace | CH |

the sera through which the two different actions are produced. I conceive that the antilysin exists in combination with other bodies in normal sera, and that part of it is easily extracted from this combination. Another and larger part, possibly, is liberated when the serum is heated to 50° C. All is set free when the temperature is raised to 60° C. The quantity which is readily yielded suffices to neutralize the complement which reacts with the amboceptors of normal sera, but is insufficient to neutralize the complement which reacts with the amboceptors of immune sera. At 50° C. a sufficiency of the antilysin is liberated to neutralize all the complement in the native sera, hence this inactivation; but as it rarely happens that a quantity in excess of this is liberated, sera heated to this temperature tend not to be antilytic. Since at 60° C. all antilysin is rendered available, the antihæmolytic action is exhibited at its height. Pe the spontaneous disappearance of complement is caused by liberation of the antilysin in the shed blood.

Another possible explanation should be considered. complement-antilysin is reversible. In the case of n serum the degree of reversibility is small; but the large nu of amboceptors contained in immune sera may *through* action possess the power of splitting the loose compound appropriating the complement.

It is desirable to study more clearly the neutralizing *acti* heated dog serum upon fresh dog serum-hæmolysis of sheep ox corpuscles. Samples of dog serum were heated *respecti* to 50°, 56°, 70°, and 90° C. In the case of the last two speci the serum was diluted with double the volume of 0.9 per c salt solution. In the tests which follow guinea-pig complem was employed. The heated serum and complement were mixed and kept for one hour at 37° C.; the corpuscles were added, and after another half hour the normal and immune s were introduced. The total volume was in each test *three cu* centimeters, and the final reading was made at the end of t hours standing at 37° C. Table V gives the results, *which may* expressed as follows: At 50° C. dog serum does not develop t antagonistic properties; at 56° and 70° C. the serum is render highly antagonistic—anticomplementary against the sever combinations employed. Heated to 90° C. the serum loses th antihæmolytic action. Since serum complements and *amboceptors* are destroyed by temperatures of 70° C. the antilytic action here described cannot be explained by supposing the *production* of complementoid or amboceptoid. The disappearance of anticomplementary action in serum heated to 90° C. may, possibly, be explained by supposing (1) destruction, (2) the formation of a new fixed compound of antilysin and serum constituent, (3) the masking of anticomplementary by auxilytic action. Since, as will be shown later in this paper, I have succeeded in separating in an active state the anticomplementary *substance*, and since it shows, in this form, marked thermostability, the first supposition may be dismissed. To decide between *the last* possibilities is less easy, but the following facts may be ed: Sera heated to from 80° to 90° C. becomes auxilytic; when deprived of lipoids, heating to these temperatures s not bring out this property. The progress of hæmolysis en serum heated to 90° C. is present is slow as compared with controls, a result that may be accounted for by supposing interaction of two opposing forces, anticomplementary and xilytic, which are not absolutely balanced.

Inasmuch as normal dog serum acquires when heated to °, or higher, antagonistic properties against the same rum in the fresh state, it is difficult to account for the enomenon on the basis of complementoid or amboceptoid roduction.

TABLE V.

| | Normal dog serum 0.5 c.c. each. | | | | Control no serum. |
|---|---|---|---|---|---|
| | 50° C—30″ | 56° C—30″ | 70° C—15″ | 90° C—15″ | |
| nti-sheep amboceptor (goat) (50° C.) 0.4<br>uinea pig complement 0.02 | CH | none | none | CH slow | CH |
| Anti-sheep amboceptor (rabbit) (50° C.) 0.1<br>guinea pig complement 0.01 | CH | none | none | CH slow | CH |
| Anti-ox amboceptor (rabbit) (50° C.) 0.1<br>guinea pig complement 0.02 | CH | none | none | CH slow | CH |
| Normal dog amboceptor (50° C.) 0.5<br>guinea pig complement 0.4<br>guinea pig corpuscles. | CH | slight | none | CH slow | CH |
| Normal dog amboceptor (50° C.) 0.5<br>guinea pig complement 0.4<br>Sheep corpuscles. | CH | trace | slight | CH slow | CH |
| Normal dog serum 0 25<br>sheep corpuscles. | CH | trace | trace | CH slow | CH |

### ABSORPTION OF THE ANTILYSIN BY BLOOD CORPUSCLES.

Since heated serum interferes with hæmolysis when brought into fluid relation with the hæmolytic serum, the question arises whether this action is exerted only against the serum hæmolysin or whether it influences the corpuscles themselves. The e already related show, quite conclusively, that the th antilysin is anticomplementary in effect. It is to be that, under the condition of experiments given, the co and antilysin combine to form, as far as hæmolysis is co an inert compound. These effects do not indicate w the reactions the corpuscles behave in an entirely passive The next experiments to be described show that under c conditions the corpuscles may take up the antilysin, throug appropriation of which they show an increased resistan hæmolytic substances.

If blood corpuscles are suspended in serum heated fr to 70° C., at 37° C. for several, at room temperatures for eig hours, separated and washed free from serum, they will be f to have become altered in susceptibility to serum hæmol Tables V, VI, VII, and VIII give the results of three suc periments. Thoroughly washed sheep corpuscles, one c centimeter, were mixed with four cubic centimeters of the se heated normal sera and kept at 37° C. for six hours. The s arated corpuscles were washed with 0.9 per cent. salt solu thirty cubic centimeters being used at each washing. T corpuscles were then subjected to the action of anti-sheep se (inactive) and guinea-pig complement (Table VI), or fresh rab serum (Table VII), or fresh dog serum (Table VIII).

TABLE VI.

| Anti-sheep ambo-ceptor (50°C.) 0.1 c.c. | Normal sera used for digesting sheep corpuscles. | | | |
|---|---|---|---|---|
| Quantity of complement | Rabbit No. 11 (60° C.) | Ox serum (60° C.) | Dog serum (60° C.) | Control untreated corpuscles |
| 0.15 c.c. | much H | CH | moderate H | CH |
| 0.1 " | none | CH | none | CH |
| 0.05 " | none | much H | none | CH |
| 0.03 " | none | slight | none | almost CH |
| 0.02 " | none | none | none | much H |

## TABLE VII.

| resh rabbit serum | Normal sera used for digesting sheep corpuscles. | | | |
|---|---|---|---|---|
| | Rabbit No. 12 (60° C.) | Ox serum (60° C.) | Dog serum (60° C.) | Control untreated corpuscles |
| c.c. | none | CH | CH | CH |
| " | none | much | much | CH |
| " | none | moderate | moderate | almost CH |
| 5 " | none | slight | slight | moderate |
| " | none | trace | trace | slight |

## TABLE VIII.

| resh dog serum | Normal sera used for digesting sheep corpuscles. | | | |
|---|---|---|---|---|
| | Rabbit No. 13 (60° C.) | Rabbit No. 14 (60° C.) | Ox serum (60° C.) | Control untreated corpuscles. |
| .2 c.c. | CH | CH | CH | CH |
| .1 " | moderate | much | CH | CH |
| .07 " | none | none | slight | CH |
| .05 " | none | none | slight | much |
| .04 " | none | none | trace | slight |
| .03 " | none | none | none | trace |
| .02 " | none | none | none | none |

## TABLE IX.

| | Temperatures to which the normal dog serum was heated before being employed for digesting corpuscles from the same blood. | | | | | |
|---|---|---|---|---|---|---|
| | Fresh | 50° C | 56° C | 70° C | 90° C | Untreated corpuscles |
| -dog amboceptor<br>0.05 c.c.<br>ea pig complement<br>0.02 c.c. | CH | CH | slight H | trace H | slight H. | CH |

## TABLE X.

| Amount of Serum | Normal dog serum (56° C.) | | Normal rabbit serum (56° C.) | |
|---|---|---|---|---|
| | Digested | Not digested | Digested | Not digested |
| 0.5 c.c. | CH | none | CH | none |
| 0.3 " | " | " | " | none |
| 0.2 " | " | " | " | trace |
| 0.1 " | " | trace | " | slight |
| 0.05 " | " | almost CH | " | almost CH |
| 0.03 " | " | CH | " | CH |
| 0.02 " | " | " | " | " |
| ntrol, no serum | CH | CH | CH | CH |

TABLE XI.

| Amount of digested serums. | Dog serum 56° C. | | | Sheep serum 56° C. | | | Ox serum 56° | |
|---|---|---|---|---|---|---|---|---|
| | Rabbit cells | Ox cells | No digestion | Rabbit cells | Ox cells | No digestion | Rabbit cells | Ox cells |
| 0.4 c.c. | slight | CH | none | CH | CH | none | much | CH |
| 0.3 c.c. | moderate | CH | none | CH | CH | trace | almost H | CH |
| 0.2 c.c. | much | CH | none | CH | CH | slight | CH | CH |
| 0.1 c.c. | CH | CH | trace | CH | CH | much | CH | CH |

The tables show that sheep corpuscles treated with se heated sera have their resistance to serum hæmolysis increa and that the effect is overcome by increasing the quanti complement in the mixture (Table VII) or the quantity o normal hæmolytic sera (Tables VI and VIII). In these te the heated sera were foreign to the corpuscles; the next te were made with serum and corpuscles from the same ani Table IX shows that if dog corpuscles are digested in hea dog serum they acquire the power of resisting a combination amboceptor and complement which completely hæmolyzes treated corpuscles, and, also, that this property of protecting corpuscles is developed by a temperature of 56° C. *but not* 50° C. A second experiment with serum and corpuscles sho that at 56° C. the property is likewise developed in *this* serum its own corpuscles.

The evidences which were obtained of the absorption corpuscles of some substance present in heated serum whi interferes with serum hæmolysis would have led naturally to th ascertainment of a corresponding disappearance of the *substanc* from the sera in which the corpuscles were digested. It happene that this phenomenon was observed before the increase *in re*sistance of the treated corpuscles was discovered and the observation led to the discovery of the protection *of* corpuscles Table X shows that heated dog and rabbit sera, digested wit corpuscles, lose their antihæmolytic property. Two cubic centimeters of dog and rabbit sera were heated to 56° C. and digested with one cubic centimeter of highly condensed (equal to five cubic centimeters original blood) washed sheep *corpuscles* at or eighteen hours. The sera separated from the corpuscles ested along with the heated sera in which no corpuscles had placed. Anti-sheep amboceptor (inactive) 0.1 cubic cen- er and guinea-pig complement 0.1 cubic centimeter were as the hæmolytic combination. Similar experiments to id not always give uniform results, due doubtless to difference gree of extractions of the antilytic bodies.

e similarity of the influence of the sheep corpuscles upon the ed rabbit and dog sera in removing antilytic bodies may be n to indicate that the removal of the antilysin from the sera t an act of elective absorption, and hence that these antilysins probably not specific bodies. The experiment summarized Table XI proves conclusively that any kind of corpuscles deprive the heated sera of the protective property. Normal , sheep, and ox sera were heated to 56° C. and two cubic centi- ers of each were treated with one cubic centimeter of con- sed rabbit corpuscles. Heated dog and sheep sera were o treated with ox corpuscles. The separated sera were ted for antihæmolytic power.

### EXTRACTION OF ANTILYSIN FROM SERUM AND CORPUSCLES.

The next step was to obtain, if possible, this thermostabile ticomplementary substance in a state of greater concentration. he earlier work of Landsteiner and v. Eisler, and Bang and orssman, and my own observations indicated that the body robably occurred among the lipoid constituents of the blood. t was, therefore, among these that I sought it. The method hich I employed does not differ essentially from the extraction method of Bang and Forssmann. I employed the separated serum and corpuscles for my work. Two hundred cubic centimeters of dog and sheep blood were shaken separately with 400 cubic centimeters of ether in a shaking apparatus, and the ethereal extracts separated and dried. These were now extracted with boiling benzol and the residue discarded. The benzol extract was dried and extracted, in turn, with aceton in the cold. Two fractions were obtained: the insoluble fraction consisting chiefly of lecithin and other phosphatids, and the soluble fracti sisting of cholesterin, fatty acids, neutral fats, and p other less defined bodies. While the reaction of the i fraction was neutral, the reaction of the soluble fracti acid. The fractions deprived of aceton were emulsified i cubic centimeters each of saline solution. The insoluble exercised no protective or anticomplementary action; was discarded. The soluble fraction, on the other hand, powerful protective activities that were not reduced by fil through compressed filter paper which removed *susp* particles. For the sake of brevity I will speak from now this saline extract as "protectin," the effects of *which I proce* to compare with those of the heated sera.

## ANTICOMPLEMENTARY AND NON-SPECIFIC ACTIONS OF PROT

The next step was, therefore, to expose susceptible corpuscles to the action of a hæmolytic combination *in the* ence of "protectin" from sera and cells and to compare effects with suitable controls. For the *experiments* corpuscles, anti-sheep serum (goat, inactivated at 50° C. cubic centimeter and guinea-pig complement 0.1 cubic centim were employed. Tables XII, XIII, and XIV show *the res* which may be stated as follows: Protectin prevents hæmo of sheep corpuscles quite as completely as do heat*ed sera* agrees with the heated sera in failing to exhibit evidences specific activity. I now proceeded to ascertain *whether* action was upon the complement of the hæmolytic combinat or upon some other constituent. Table XV gives the res obtained and is taken to indicate action upon the compleme of the hæmolytic complex. An identical result was obtain with sheep protectin.

## ABSORPTION OF PROTECTIN BY CORPUSCLES.

Since up to this point the protectin solution acted in a mann which was not to be distinguished from the heated sera, a te of the power of corpuscles to absorb the active agent *from t* on was next made. Table XVI shows that in this respect otectin solution is comparable with the heated sera.

## XTRACTION OF PROTECTIN FROM DRIED AND HEATED SERA AND CORPUSCLES.

ere was available for preparing the protectin a large quantity ied sera and corpuscles. These products had been collected, d, and subjected for thirty minutes to a temperature of C. I had at my disposal the sera and corpuscles of dog, ox, horse which, after the treatment just mentioned, had been hand for nearly two years. The several products were vdered and extracted in a shaker with ether (forty-eight hours), er which the further steps were identical with those already en. I was thus enabled to obtain relatively large quantities the protectin fraction which was tested against various normal d immune sera with the results already given. By means of any tests I came to the conclusion that both blood serum and rpuscles, of all the mammals studied by me, contain a body hich has the property of protecting corpuscles of different ammals from the effects of serum hæmolysins, and that this ody is highly stable and very resistent to heat.

TABLE XII.

| Amount of protectin in c.c. | Source of protectin | | | |
|---|---|---|---|---|
| | Dog | | Sheep | |
| | Serum-protectin | cell-protectin | Serum-protectin | Cell-protectin |
| 0.5 | none | none | none | none |
| 0.4 | | | “ | |
| 0.3 | | | trace | |
| 0.2 | trace | | slight | trace |
| 0.15 | “ | | “ | “ |
| 0.1 | slight | | much | slight |
| 0.07 | moderate | trace | almost CH | “ |
| 0.05 | almost CH | slight | CH | almost CH |
| 0.03 | CH | “ | | CH |
| 0.02 | | almost CH | | |
| 0 (control) | | CH | | |

## TABLE XIII.

Sheep corpuscles were to be protected from fresh **active immun** (goat).

| Amount of anti-sheep serum | Control (no protectin) | Sheep cell-protec |
|---|---|---|
| 0.3 c.c. | CH | trace |
| 0.2 " |  | none |
| 0.15 " |  |  |
| 0.1 " |  |  |
| 0.07 " | much H |  |
| 0.05 " | slight |  |
| 0.04 " | trace |  |
| 0.03 " | none |  |

## TABLE XIV.

Sheep corpuscles were to be protected from fresh **normal dog serum**

| Amount of dog serum | Control (no protectin) | Sheep cell-protectin 0.4 c.c. | Dog cell-prot 0.4 c.c. |
|---|---|---|---|
| 1.0 c.c. | CH | moderate | slight |
| 0.7 " |  | slight | " |
| 0.5 " |  |  |  |
| 0.5 " |  | none | none |
| 0.3 " |  |  |  |
| 0.2 " |  |  |  |
| 0.1 " |  |  |  |
| 0.07 " |  |  |  |
| 0.05 " | slight |  |  |

## TABLE XV.

1) Dog serum-protectin 0.3 c.c. + g. p. complement 0.1, 1 hour at 37° C
+ anti-sheep amboceptor 0.2 c.c. + corpuscles = no H
2) Dog serum-protectin 0.3 c.c. + g. p. complement 0.2 c.c., 1 hour at 37° C
+ anti-sheep amboceptor 0.2 c.c. + corpuscles = CH
3) Dog serum-protectin 0.3 + g. p. complement 0.1 c.c., 1 hour at 37° C
+ anti-sheep amboceptor 0.4 c.c. + corpuscles = no H
4) No protectin (control) + g. p. complement 0.1 c.c., 1 hour at 37° C
+ anti-sheep amboceptor 0.2 c.c. + corpuscles = CH

## TABLE XVI.

1) Sheep corpuscles 0.5 c.c. + sheep cell-protectin 4.0 c.c. kept 4 hours at 37° C., after which the corpuscles were separated from the fluid and washed in 0.9% saline solution.

This will be called "sheep cell-protectin treatment."

2) Sheep corpuscles 0.5 c.c. + dog cell-protectin 4.0 c.c. kept 4 hours at 37° C.

which the corpuscles were separated from the fluid and washed in
% saline solution.
vill be called "dog cell-protectin treatment."
nolytic complex was: anti-sheep amboceptor (50° C.) 0.1 c.c. and
nea pig complement 0.02 c.c.
servation: 30 minutes at 37° C.
"'Sheep cell-protectin treatment" plus hæmolyser give no hæmolysis.
og cell-protectin treatment" plus hæmolyser give slight hæmolysis.
treated sheep corpuscles plus hæmolyser give complete hæmolysis.

s condition of thermostability is one of its most interesting es, and brings it, at first sight, into contrast with the ther-abile, so-called protective principles of normal serum

TABLE XVII.

| mperature | Duration of heating | Color | Reaction | Antihæmolytic power |
|---|---|---|---|---|
| ° C | 1 min. | water clear | acid | no H |
| | 5 min. | " | " | |
| | 15 min. | " | " | " |
| | 30 min. | " | " | " |
| | 60 min. | " | " | " |
| | 90 min. | " | " | trace H |
| | 120 min. | trace yellow | neutral | slight H |
| o° C | 10 min. | light yellow | " | " |
| 5° C | 10 min. | light amber | alkaline | moderate H |
| o° C | 10 min. | amber | " | " |
| nheated | | | acid | no H |

emarks: To raise the temperature from 100° C. to 120° C. 40 minutes, from ° to 135° C. 10 minutes, and from 135° to 150° C. 10 minutes are required. To l the antoclave from 150° to 100° C. it took about 60 minutes.

e question presents itself whether bodies which exhibit such arked differences in properties can possibly be identified with ch other. My own views, already outlined on pages 732 and 6, make it possible for me to conceive readily that they are entical. It will be unnecessary to repeat here what I have lready stated regarding my reason for thinking that loss of pro-ective property of serum when heated to 90° C. is not due to estruction of the protective body, but to development of uxilytic compounds or recombination of the former body.

My next experiment was to ascertain the effects of high tem-eratures upon the protectin solution. Dog corpuscle protectin n 0.9 per cent. salt solution was placed in tubes, which were hermetically sealed. Table XVII shows the temper which the tubes were heated and the alteration in appearances which resulted. The antihæmolytic po the solutions was tested against anti-sheep serum 0.2 cubic centimeter, guinea-pig complement 0.05 cubi meter, and sheep corpuscles. The controls give co hæmolysis. The quantity of the protectin used in eac was 0.2 cubic centimeter. From the table it will be see the solution is highly resistant to heat, but that a dimi in activity, associated with changes in reaction, occu the higher temperatures.

### SUMMARY.

Blood serum contains normally certain principles whic hibit serum hæmolysis by interfering with the action of c plement. In the case of most sera the anticompleme action appears only after heating to 56° C. or higher.

The inhibiting action of this principle is directed against a as well as native complements, and is non-specific in chara

It would appear as if the inactivation of serum at 50° C thereabouts were due to a partial liberation of the anti principle which just about suffices to neutralize the action of native complement. As the temperature of the serum is rais up to a certain point, more and more of the antilysin is set until the serum comes to contain an overplus, in exces the quantity neutralizing its own complement, which is ca ble of interfering with the action of additional native or al complements.

Serum heated to 90° C. is less antilytic than serum heated 70° C., either because the antilysin has entered into new, mo stable compounds which prevent its action, or, as is more probabl because of the liberation from the serum of a second group o principles in themselves hæmolytic directly, or indirectly b increasing the power of serum hæmolysins (auxilysins). Th latter action tends to mask and to suppress the inhibitor influences of the antilysin.

The antilysin is removed from serum by digestion with man of blood corpuscles which, apparently, absorb the principle. e, by reason of this treatment, the serum is deprived of its itory power, the corpuscles have acquired greater resistance rum hæmolysins.

y treating blood serum and corpuscles with ether the antilysin be extracted. The ethereal extract, freed from lecithin and ain related bodies, contains the antilysin in a concentrated not in a pure form, which can now be taken up in saline tion in which it dissolves.

he saline solution of the antilysin, which for convenience has n denominated "protectin," behaves in all respects like native antilytic sera except that it is uninfluenced by nperatures of 90° C. or even higher temperatures. Protectin hibits serum hæmolysis directly by neutralizing complement nd indirectly after absorption by corpuscles by increasing their sistance. Hence it is highly probable that the antilytic principle of heated serum and protectin extracted from serum and rpuscles are the same substance.

Thermostability is one of the characteristic properties of rotectin. Serum and corpuscles, first dried, may be heated to 50° C. without losing the protecting principle, and the principle ersists in such heated products for at least two years. Protectin n solution is unaffected by a temperature of 100° C. maintained for one hour, and suffers only slight reduction in activity at the expiration of two hours; while temperatures ranging from 135° to 150° C. bring about marked reduction in protective power. As these alterations are produced by high temperatures the reaction of the solution changes from acid, through neutral, to alkaline.

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